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CEREAL CHEMISTRY

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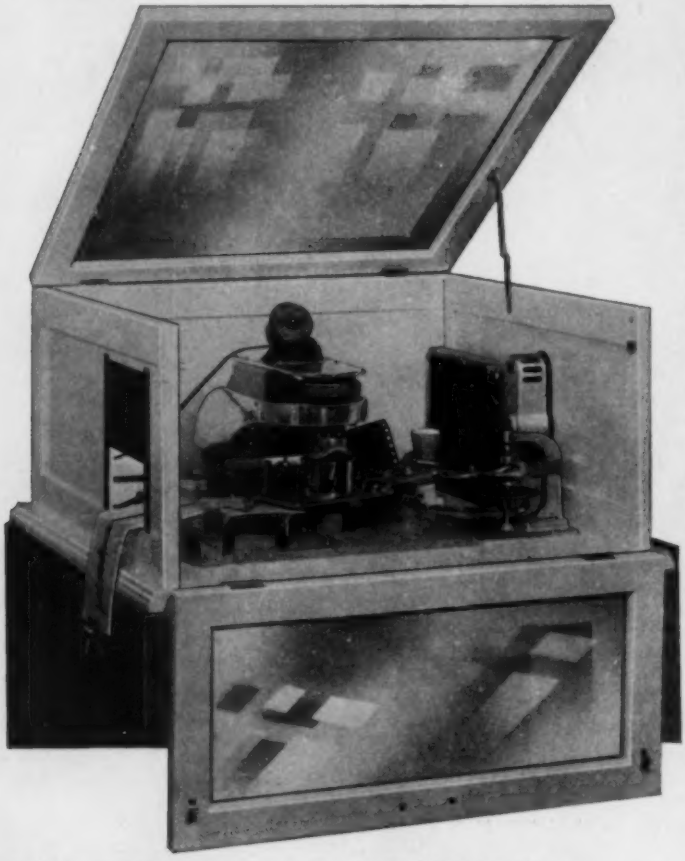

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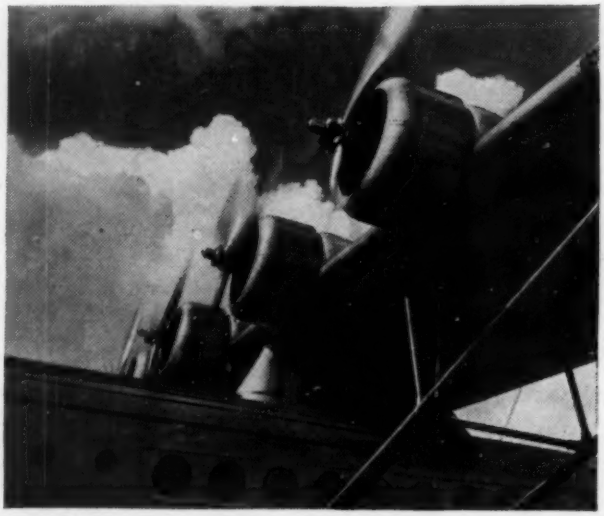

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CEREAL CHEMISTRY

VOL. XIX MARCH, 1942 No. 2

A COLLABORATIVE STUDY OF VARIOUS METHODS FOR ESTIMATING THE PIGMENT CONTENT OF FLOUR EXTRACTS [1]

D. S. BINNINGTON

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1941)

In accordance with the recommendation of the 1939–40 Committee on Methods of Analysis, studies upon the determination of flour pigments have been extended to a collaborative investigation of various procedures for estimating the pigment content of flour extracts.

In this study, variables associated with preparation of the extracts themselves were eliminated by supplying the collaborators with aliquot portions of large samples of flour extracts prepared in one laboratory. In the majority of cases, naphtha + alcohol (93 : 7) was employed as solvent, since Ferrari and Croze[2] have shown that flour extracts prepared with this solvent are stable at room temperature for at least nine months if protected from excessive exposure to daylight. However, since several collaborators possessed instruments calibrated only for water-saturated butanol, extracts were prepared with this solvent also. As a check upon the stability of these extracts, samples were stored in clear glass bottles in a dark cupboard at room temperature for a period of 35 days. No change in pigment concentration could be detected.

The results obtained by eight collaborators using naphtha-alcohol extracts are given in Table I and the means for methods in Table II.

These results in general show good agreement, particularly when the wide variety of instruments and methods employed is taken into consideration. The spectrophotometric results show greater divergence than might be anticipated. Collaborators 5 and 6, however, obtained results in excellent agreement using full spectrophotometric

[1] Subcommittee Report, 1940–41 Committee on Methods of Analysis, Paper No. 26, Journal Series, General Mills, Inc., Research Laboratories.

[2] C. G. Ferrari and Alice B. Croze: A flour extract as a standard in the colorimetric estimation of flour color, Cereal Chem. 11: 511–514 (1934).

TABLE I

PIGMENT CONTENT OF FLOUR EXTRACTS PREPARED WITH NAPHTHA-ALCOHOL SOLVENT—ALL METHODS

Collaborator	Flour pigment—Samples No.			Method
	1	2	3	
	ppm	*ppm*	*ppm*	
3	1.03	1.89	3.10	Spectrophotometric
4	0.94	1.93	3.46	"
5	0.99	1.98	3.14	"
6	1.01	2.13	3.20	"
1	1.05	2.16	3.27	Evelyn photoelectric colorimeter
7	1.05	2.09	3.17	" " "
8	1.10	2.17	3.24	" " "
2	1.05	2.16	3.27	Visual
Mean	1.02	2.04	3.22	
Range	0.16	0.24	0.36	

Equipment

3 Bausch & Lomb polarization photometer with Hg vapor light source and filter isolating 435.8 mμ line.
4 Bausch & Lomb spectrophotometer with white light source, readings taken at 435.8 mμ, 0.025 mm slit width.
5 Bausch & Lomb spectrophotometer with Hg vapor light source, readings taken at 435.8 mμ.
6 König-Martens spectrophotometer with Hg vapor light source, readings taken at 435.8 mμ.
2 Method of Geddes, Binnington, and Whiteside, Cereal Chem. **11**: 1–24 (1934).
1, 7 & 8 Common standardization—Binnington, Cereal Chem. **17**: 639–645 (1940).

TABLE II

PIGMENT CONTENT OF FLOUR EXTRACTS PREPARED WITH NAPHTHA-ALCOHOL SOLVENT—MEANS OF METHODS

Method	Flour pigment—Samples No. 1	2	3
	ppm	*ppm*	*ppm*
Spectrophotometric	0.99	1.98	3.22
Evelyn photoelectric colorimeter	1.07	2.14	3.23
Visual	0.95	2.00	3.20

equipment as against the abridged forms employed by Collaborators 3 and 4.

The results obtained by the collaborators using water-saturated butanol extracts are detailed in Table III.

In considering these results, it should be noted that while prepared from the same flours as the naphtha-alcohol extracts, different sample-to-solvent ratios were employed and, therefore, no quantitative relation exists between the two sets of samples. A somewhat greater range is evident in this series of tests even when the results of Col-

laborators 9 and 10 are excluded. This range would be considerably less if the spectrophotometric results obtained by Collaborator 3 were omitted. The best standard for comparison in this series is probably the spectrophotometric results obtained by Collaborator 6. Such a comparison indicates excellent agreement within this group, exclusive of Collaborator 3.

The very low results reported by Collaborators 9 and 10 are apparently due to the use of beta-carotene for calibrating the photoelectric instruments employed and emphasize the importance of a uniform mode of standardization.

TABLE III

PIGMENT CONTENT OF FLOUR EXTRACTS PREPARED WITH WATER-SATURATED BUTANOL—ALL METHODS

Collaborator	Sample			Method
	1	2	3	
	Flour pigment ppm			
3	1.03	1.94	2.46	Spectrophotometric
6	1.24	2.16	2.57	"
7	1.39	2.26	2.62	Evelyn photoelectric colorimeter
6	1.39	2.26	2.62	" " "
9	.86	1.56	1.87	KWSZ photometer
10	.60	.95	1.10	" "
3*	1.33	2.27	2.80	Visual (Aminco photometer)
Mean (excluding 9 and 10)	1.28	2.18	2.61	
Range (excluding 9 and 10)	.36	.33	.34	

9 and 10 Calibrated against beta-carotene.
3* Calibrated against spectrophotometer.

Discussion

The solvents employed for flour pigment determinations extract a complex mixture of pigments comprising alpha and beta carotenes, xanthophyll and its esters, and tricin and other pigments of the flavone type. Since no single pigment is determined as such, the method must be classed as an empirical one. Unquestionably, certain of the individual pigments such as carotene and xanthophyll could be estimated by methods similar to those developed for alfalfa and other feeds containing pro-vitamin A, but no justification is evident for such a course, since the primary object of a flour pigment determination is to secure an estimate of the *total* pigment present as an index of the yellow color of the flour.

In estimating the pigment content of flour extracts by methods other than spectrophotometric, some form of standardization or cali-

bration is essential. This point is of particular importance when photoelectric colorimeters are employed, since some form of filter isolating a narrow region of the spectrum must be used. If such calibration is made with standards differing widely in their spectral properties from those of flour extracts, as is the case with pure carotene, erroneous results are inevitable. It is therefore recommended that flour extracts of known pigment content determined spectrophotometrically alone be employed as a basis for standardizing other techniques.

In the past, the terms carotene and carotinoid pigments have been rather loosely used to indicate the total pigment extracted from a flour by a suitable solvent. In view of the empirical nature of the determination, these terms are entirely too specific and it is, therefore, further recommended that the more accurate term "flour pigments expressed as carotene" be substituted.

Summary

Methods of estimating the pigment content of flour extracts have been submitted to collaborative study in the instance of three extracts and ten collaborators.

Three different modifications of the spectrophotometric procedure, two types of photoelectric colorimeters, and two visual procedures were employed using both naphtha-alcohol and water-saturated butanol solvents.

In general, the results obtained were in good agreement with the exception of two operators using photoelectric instruments calibrated against beta carotene.

The empirical nature of this determination is pointed out and it is, therefore, recommended that the spectrophotometric method alone be employed as a basis for standardizing other techniques.

The use of the term "flour pigments expressed as carotene" is suggested in preference to "carotene" or "carotinoid pigments."

Acknowledgments

The assistance of Mark A. Barmore, R. L. Cunningham, W. F. Geddes, Willis S. Hutchinson, Harold Johannson, R. K. Larmour, V. W. Morris, A. G. O. Whiteside, and W. W. Worzella, who collaborated in this study, is gratefully acknowledged.

THE NICOTINIC ACID CONTENT OF CEREAL PRODUCTS

JAMES M. THOMAS, ALBERT F. BINA, and ELMER B. BROWN

Anheuser-Busch, Inc., Laboratories, St. Louis, Missouri

(Received for publication August 14, 1941)

In a previous communication Bina, Thomas, and Brown (1941) described a procedure for the determination of nicotinic acid applicable to yeast, flour, bread, and cereal products. This method consists essentially in subjecting the sample to be tested to the action of a diastatic reagent (takadiastase) under suitable conditions for diastasis so as to liquefy and saccharify the starchy material in the preparation of the extract. The nicotinic acid content of the extract is then determined after treatment with *p*-aminoacetophenone and cyanogen bromide by reading the color by means of the fluorophotometer. The nicotinic acid content is then evaluated by applying the procedure described in our previous communication.

It was also stated in the previous publication (see "Literature Cited") that other procedures had been studied and found unsuitable for the determination of nicotinic acid in flour, bread, and cereal products because of the semisolid mass resulting from the treatment of this type of material and other interfering products. It was also observed that with the reagents employed, the turbidity and substances other than nicotinic acid influenced the determination to such an extent that the results were erratic and unreliable for our purpose. This was especially true when either aniline, as used by Melnick, Oser and Siegel (1941), metol or acetone, as employed in the procedure of Bandier and Hald (1939), and, in some instances where *p*-aminoacetophenone was employed. It was observed, however, that in the use of *p*-aminoacetophenone no trouble is experienced if the *p*-aminoacetophenone solutions are made up weekly, but may develop with solutions of a month or more of age.

The erratic results obtained when aniline was used as one of the reagents in the procedure may be due in part to the fact that we did not use freshly distilled aniline. In a former research (Nau, Brown, and Bailey, 1925) where aniline was used, erratic results were obtained unless the aniline was distilled daily. Even with this precaution these authors found aniline to be very reactive, giving rise to various anilides if di- or tri-basic acids were present in the reaction mixture.

Experimental

In Table I are assembled the data on a representative sample of a typical Kansas winter wheat mix and on the various milling products

obtained from it in the milling process. These samples were furnished by Mr. Whinery of the Rodney Milling Company. The ash, protein, and moisture were determined by Mr. Whinery and checked by us. The nicotinic acid was determined by the procedure described in our previous communication to this journal, reference to which has been made.

TABLE I

KANSAS WINTER WHEAT MIX

Sample	Ash	Protein	Moisture	Nicotinic acid
	%	%	%	*mg per lb*
Wheat	1.52	13.00	11.53	6.01
Patent	0.38	11.52	13.68	1.58
Straight	0.43	12.08	13.63	2.03
1st clear	0.67	14.00	13.47	4.15
2nd clear	0.88	14.20	13.30	7.27
Red dog	2.17	16.54	12.58	10.20
Shorts	3.94	17.62	13.00	13.75
Bran	5.89	15.65	13.98	20.43
Germ [1] (defatted)	3.54	30.90	11.30	18.26

[1] The wheat germ was the only portion that indicated interfering substances of appreciable extent as affecting the analysis. This was noticeable in the orange color tint produced and the abnormally high blanks obtained when the determinations were made without defatting. By defatting the germ with ether, this interference disappeared, giving a normal low blank and characteristic color free from the orange tint. It is our opinion that the lower figure obtained on the defatted material is the correct one for this particular sample and the difference between 24.13 obtained on the original and 18.26 on the defatted material represents the removal of interfering substances rather than the loss of nicotinic acid by the ether extraction. The wheat germ from the spring mix gave the same nicotinic acid content on both the original and defatted material, indicating no loss of nicotinic acid by the extraction, and no interference that was not removed by the blank. The only difference was in the size of the blank values. The ash, protein, moisture and nicotinic acid recorded in the defatted germ column are calculated on the original sample and not on the defatted germ basis.

In Table II are assembled the data on a representative sample of a typical spring wheat mix and the various milling products obtained from it. These samples were supplied by Dr. John Andrews of General Mills, and the nicotinic acid determined in the same manner as with the Kansas winter wheat mix throughout.

TABLE II

SPRING WHEAT MIX

Sample	Ash	Protein	Moisture	Nicotinic acid
	%	%	%	*mg per lb*
Wheat	1.74	14.10	11.7	9.56
Patent	0.41	12.97	11.4	3.19
1st clear	0.77	16.24	10.7	8.02
2nd clear	2.20	19.71	11.8	8.98
Red dog	3.68	18.78	9.9	14.70
Shorts	5.20	16.79	11.0	17.82
Bran	6.31	16.04	9.9	31.22
Germ (defatted)	4.44	27.53	9.8	28.81

It is apparent that the milling separations used on these two wheat mixes were not identical, and hence not completely comparable for the mill-stream products obtained. However, the wheat samples were ground by us by means of a hammer mill, and the nicotinic acid values obtained are comparable on the wheats. These results show that this spring wheat had 1.59 times the amount of nicotinic acid found in the winter wheat sample. Stated in different terms, the nicotinic acid content of the winter wheat was 62.9% of that of the spring wheat.

In order to check as closely as possible on the applicability of the method, as applied to these products, Dr. Andrews informed us as to the percentages of the total wheat these samples respectively represent. From these percentages and from the amounts of nicotinic acid found in the whole wheat and the various milling products obtained from it, we have calculated the amounts removed by each mill fraction (Table III).

TABLE III

NICOTINIC ACID IN MILL FRACTIONS

Sample	Yield	Nicotinic acid	Nicotinic acid in fractions	
	%	*mg per lb*	*mg*	%
Wheat	100	9.56	9.560	100
Patent	63	3.19	2.009	22.00
1st clear	7	8.02	0.561	6.14
2nd clear	4.5	8.98	0.404	4.43
Red dog	4	14.70	0.588	6.44
Shorts	12.3	17.82	2.191	24.00
Bran	9	31.22	2.799	30.66
Germ	0.2	28.83	0.576	6.31

Total nicotinic acid by calculation of mill streams	9.128
Total nicotinic acid found by analysis on actual wheat used in the milling	9.560

The small difference between the total nicotinic acid content calculated from the results obtained by assay of the mill streams and the nicotinic acid found in the whole wheat, indicates that the method of assay is consistently accurate for milling products and cereal grains. It would also indicate that the percentages of the mill streams obtained from Dr. Andrews are quite accurate.

In Table IV are tabulated the nicotinic acid contents of several cereal grains as determined by our procedure. Each of these was ground in a hammer mill through the same mesh screen, care being taken that all passed through the screen.[2]

[2] The sample of barley used was ungraded midwestern barley. The malt was No. 3 midwestern malt and bears no direct relationship to the barley sample.

TABLE IV

CEREAL GRAINS

Sample	Protein	Ash	Moisture	Nicotinic acid	
	%	%	%	*mg per lb*	*mg per lb dry solids*
Peanuts: raw	25.52	2.23	9.40	43.58	48.11
roasted	24.30	2.21	9.14	45.66	50.14
Wheat mix, spring	14.10	1.74	11.70	9.56	10.83
Wheat mix, winter	13.00	1.52	11.53	6.01	6.79
Barley [1]	10.39	2.48	11.10	6.86	7.72
Malted barley [1]	11.19	2.28	5.63	9.14	9.68
Oats	10.50	3.72	9.39	3.33	3.66
Rye	12.55	1.82	10.47	4.40	4.92
Corn (yellow)	7.50	1.76	14.34	3.17	3.70
Corn (white)	8.87	1.36	12.56	3.35	3.73
Soybean	32.64	5.21	9.99	9.04	10.04
Rice (unhulled)	5.91	4.75	12.27	5.07	5.78
Rice (brown)	7.57	1.05	11.35	3.65	4.11
Rice (polished)	7.22	0.895	12.27	2.099	2.39

[1] The analyses were made on these cereals after they were pulverized in the Wiley Laboratory Mill, Intermediate Model.

The nicotinic acid contents of these cereals were calculated on the moisture content, as received, and also on a moisture-free basis. The results in the moisture-free column therefore afford reliable comparisons of the amounts of nicotinic acid contained in the different cereals.

TABLE V

FLOURS

Sample	Ash	Protein	Moisture	Nicotinic acid
	%	%	%	*mg per lb*
WHOLE-WHEAT FLOURS				
Purina	1.65	14.00	10.0	6.23
Staff	1.62	15.27	11.7	7.09
Spring	1.75	14.60	11.8	7.44
WHITE FLOURS				
Rodney	0.37	11.35	15.0	1.55
Gibralter	0.39	11.74	15.0	2.86
Spring	0.41	12.86	12.9	3.37
Dulle	0.40	12.00	15.0	3.85

Table V contains the data on the nicotinic acid contents of different flours. Some of the flour samples were obtained from bakeries and are typical of flours used in bread manufacture.

The whole-wheat flours did not show as high a nicotinic acid

content as did the ground whole wheats used. This would indicate that some of the higher nicotinic acid portions of the whole wheat are eliminated in the milling process.

Table VI gives the nicotinic acid content of several samples of bread, including whole-wheat bread, white bread and enriched white bread. The nicotinic acid content is calculated to a 34% moisture basis. The two samples under "white bread" differ only in the flour used in the same bakery. The "enriched bread" samples were purchased on the market in different sections of the country and are representative of the nicotinic acid content of commercial enriched bread. The nicotinic acid content of the enriched white breads is considerably higher than for whole-wheat bread.

TABLE VI

BREADS

	Nicotinic acid *mg per lb*
WHITE BREAD	
Bread No. 1	1.90
No. 2	2.42
WHITE BREAD (ENRICHED)	
Sample A	8.50
B	9.69
C	9.96
WHOLE-WHEAT BREAD	
Cap sheaf	4.08
Staff	5.25

The nicotinic acid content of yeast is relatively high, but varies somewhat according to culture and environmental conditions. Regular baker's yeast normally contains from 40 to 70 mg per pound of nicotinic acid as received by the baker and approximately 3⅓ times this amount on a dry basis. Enriched yeast is standardized to contain not less than 500 mg of nicotinic acid per pound. Table VII contains data on the nicotinic acid content of different yeast samples.

Discussion

In the preparation of an extract of a cereal product for analysis it is difficult to determine the completeness of the extraction procedure for bringing the material into solution. We cannot state positively

TABLE VII

YEASTS

	Nicotinic acid	
	mg per lb. 70% moisture basis	*mg per lb. dry basis*
BAKER'S YEAST		
No. 1	58.5	195
2	57.3	191
3	56.1	187
BAKER'S YEAST (ENRICHED)		
No. 1	549.9	1833
2	558.3	1861
3	561.0	1870

that our extraction procedure removes all of the nicotinic acid from the products we have analyzed, although the results are reproducible on all of the materials analyzed and complete recovery of the superimposed nicotinic acid is obtained. The fact that the total of the nicotinic acid contents determined in the various ingredients used in bread is found to agree with the analysis of the bread itself, leads us to believe the extraction is complete.

We are aware of the possibility of interference from compounds other than nicotinic acid, which are not biologically active but which may produce a color reaction similar to that produced by nicotinic acid in the presence of an aromatic amine and cyanogen bromide. The results obtained lead us to conclude that cereal products do contain such compounds. These compounds are not completely removed by decolorizing the aqueous solution and they may in turn affect the color determination, if the color produced is read in the aqueous extract. We believe that the use of an organic solvent, for example ethyl acetate, as recommended by Harris and Raymond (1939), which will extract the color complex produced by the König (1904) reaction from the other interfering substances that may be present in the original extract, gives a better procedure for eliminating interfering substances than the complicated decolorizing techniques. These complicated decolorizing procedures, as pointed out by Dann and Handler (1941), may, in addition, introduce a source of error due to loss of nicotinic acid through adsorption and elution.

p-aminoacetophenone has proved the most reliable of the aromatic amines. It is more stable, the color complex produced with it is readily and completely extracted by ethyl acetate, and the color is

quite stable to light. None of the other amines tried met these important requirements.

Summary and Conclusions

We have applied our method for the determination of nicotinic acid to a number of cereal products. The method includes liquefaction of the sample by the action of a diastatic reagent, extraction of the vitamin by autoclaving a water suspension of the material, conversion of the starch to sugar, separation and hydrolysis of the extract with dilute hydrochloric acid, adjustment of the pH for proper color development with *p*-aminoacetophenone, cyanogen bromide, and nicotinic acid, extraction and separation of the color complex from extract with ethyl acetate, and the determination of the color in the ethyl acetate phase by means of the fluorophotometer, involving comparison with a known standard of nicotinic acid similarly treated.

Products like wheat germ contain large amounts of material extractable with fat solvents. These products give high blank values and may produce colors that interfere with fluorophotometric readings. These substances can be removed by ether extraction of the original sample. The above procedure eliminates interference from color-producing compounds contained in cereal products that would otherwise produce erratically high results if not eliminated in the nicotinic acid determination.

The results reported give the nicotinic acid content of a number of cereal and baking products. The nicotinic acid content is appreciable and variable in wheat and flour, but considerably lower than the amounts reported by investigators employing other procedures.

The nicotinic acid contents of the various milling products of wheat were determined. The bran was found to contain the highest percentage, and the largest amount of this vitamin of any of the milling products. The percentage in mill products increased as the ash content increased from short patent flour to the bran. The patent and first and second clear flours combined contained approximately one-third and the mill feeds approximately two-thirds of the nicotinic acid of the wheat.

The spring wheat sample contained the largest amount of nicotinic acid per pound (10.83 mg) with soybean second. Oats contained the least amount per pound (3.66 mg) with corn almost the same (3.70 mg). Malt, barley, rye, and brown rice were between these values.

Whole-wheat flours showed less nicotinic acid than ground whole wheats, indicating they had undergone some refinement in the milling process.

The nicotinic acid content of whole-wheat bread, *i.e.* whole-wheat bread made from whole-wheat flour, was above the minimum requirement for enriched bread, but considerably below the average nicotinic acid content found in enriched white bread.

The nicotinic acid content of baker's yeast showed from 55 to 60 mg per pound on a 70% moisture basis, and 190 mg per pound on a dry basis. Enriched baker's yeast showed approximately 550 mg per pound on a 70% moisture basis.

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THE EVALUATION OF MALT FOR USE AS A FLOUR SUPPLEMENT [1]

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The use of germinated cereals for supplementing bread flours has been practiced for many years. Baker and Hulton (1908) described this use as "common" as of that date. These authors, by their proposal of an "enzyme theory" of flour strength, probably were the first to recognize the significance of flour enzymes, both amylolytic and proteolytic, in baking. They postulated that the beneficial effect of malt supplement was due to action of the "starch liquefying enzyme" provided by this means.

During the years following 1908 many investigations have been made of malt supplementation and the manner in which flour diastatic activity influences loaf quality. Space does not permit an adequate review of the extensive literature. The reader is referred to the review paper of Hesse (1935) and to papers by Rumsey (1922), Collatz (1922), Green (1934), Read and Haas (1934, 1936), Landis and Frey (1936), and Hildebrand and Geddes (1940). As summed up by Freeman and Ford (1941) the main reasons for the present extensive use of malt supplement are (1) to increase gas production, (2) to improve the crust color, (3) to increase the moistness of the crumb, (4) to impart additional flavor.

Neumann and Salecker (1908) early observed that increases in loaf volume or in gas production were not closely correlated with the "Lintner value" of malt supplement. Following this observation the belief has developed that the factor in malt supplement responsible for increased loaf volume is the alpha component of malt amylase. Munz and Bailey (1937) found that alpha- rather than beta-amylase was responsible for changes in dough mobility and stimulation in loaf volume. Sandstedt, Jolitz, and Blish (1939) postulated that the improving effect of malt on flour doughs was due to the alpha component of malt amylase. Freeman and Ford (1941) conclude that any method for evaluating malts for flour supplementation must be capable of distinguishing between different alpha-amylase contents.

Practically all the workers have emphasized the value of malt supplement in preventing "yeast starvation" and maintaining gas production, particularly during the "proof" period of bread dough.

[1] No. 287, Journal Series, Nebraska Agricultural Experiment Station.

It is notable that most of the researches have been conducted under conditions where sugar content of the dough could well be a factor limiting loaf volume. Blish and Hughes (1932) showed that a study of baking properties other than those related to gas production in the test loaf could have value only when this gas production is adequately maintained. Further, Sandstedt and Blish (1939) demonstrated that the use of fairly high sugar levels in the baking formula combined with proofing to height essentially eliminated sugar production as a variable influencing loaf volume.

Recently malt supplementation studies (Sandstedt, Jolitz, and Blish, 1939; Geddes, Hildebrand, and Anderson, 1941) have been conducted at high enough sugar levels in the baking formula so that it is improbable that sugar production was a factor limiting loaf volume. It is interesting that even at this sugar level (5%) Geddes, Hildebrand, and Anderson (1941) concluded (p. 57) that "the results of the baking tests clearly indicate that the amylase activity of the malted wheat flours is the only property of any significance in their utility for diastating purposes." These results at high sugar levels suggest that, in addition to the effect on gas production in doughs, diastatic supplements may have an important role in enhancing *gas retention* in the oven.

Recent methods for evaluating malt supplements have been based on the technique of Leatherock, McGhee, and Giertz (1937) for producing flours with constant autolytic diastatic values. Davis and Tremain (1938) modified this method to apply to the standardization of six-hour gas production values. Sandstedt (1938) and Hildebrand and Geddes (1940) report data confirming the value of this type of malt evaluation. These methods reflect only the value of malt supplement for enhancing gas production in a dough and supply no information regarding the possible effect on the "oven spring" indicative of gas retention in the oven.

Apparently there have been few attempts to correlate directly gassing power response from malt supplementation with loaf volume increase, or for that matter malt alpha-amylase activity with gassing power response. Geddes, Hildebrand, and Anderson (1941) observed that when flours were malted to a uniform gassing power level the resulting loaves were of uniform size. Sandstedt (1938) found fair correlation between malt alpha-amylase activity and gassing-power response. However the "alpha-amylase method" used in this investigation was the essentially unmodified Wohlgemuth procedure shown later by Sandstedt, Kneen, and Blish (1939) to be a measure not of the alpha component but of the combined actions of alpha- and beta-amylases.

Since the influence of malt supplement on the gassing power of doughs must be due to added alpha-amylase it seems pertinent to examine the relationships between malt saccharogenic activity, alpha-amylase activity, and stimulation of gas production. Further, additional information is needed regarding the ability of malt supplement to enhance *gas retention* during baking and the relationship of present methods of malt evaluation to this response. In the present investigation the alpha-amylase values reported are specific for that component and baking responses were obtained under conditions in which gas production was eliminated as a variable.

Methods

Alpha-amylase activity: Alpha dextrinogenic activity was determined by the technique of Sandstedt, Kneen, and Blish (1939). In this method an accurate evaluation of the alpha component is achieved by measuring dextrinization in the presence of an excess of the beta component. "Alpha-amylase units" are the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 30°C.

Saccharogenic activity: Saccharogenic activity was determined by the method of Kneen and Sandstedt (1941). In this method maltose production from the action of unmodified malt extract on soluble starch over a 15-minute period is measured. "Saccharogenic units" are the number of grams of soluble starch saccharified by one gram of malt in one hour at 30°C. "Lintner values" were calculated according to the technique of Kneen, Beckord, and Sandstedt (1941): Kneen-Sandstedt units $\times$ 7.5 = degrees Lintner.

Beta-amylase activity: The primary object of these studies has been to evaluate the relative importance of saccharogenic activity and of alpha-amylase activity in malt evaluation. Accordingly the amount of saccharification attributable directly to the beta component of malt amylase by itself was calculated infrequently. Where recorded, beta-amylase activity was determined by the method of Kneen and Sandstedt (1941). In this method the amount of maltose production attributable to the alpha component is determined and subtracted from the maltose produced by the action of the unmodified malt extract on soluble starch. The difference represents beta saccharification and beta-amylase units are calculated as the number of grams of soluble starch saccharified by the beta-amylase of one gram of malt in one hour at 30°C.

Gassing power: Gas production in supplemented and unsupplemented flours was measured by the technique of Sandstedt and Blish (1934). The Sandstedt-Blish "pressuremeter" was used in conjunc-

tion with doughs made from 10 g flour, 0.3 g yeast, and water and malt extract sufficient to give an added liquid volume of 10 ml. Results are given as mm of mercury measured after 6 hours' action at 30°C and in most instances are recorded as increases in gas production, *i.e.*, as the gas production attributable to the malt supplement.

Baking procedure: The baking procedure used was essentially that recommended by the A. A. C. C. The actual formula was 100 g flour, 6% sugar, 3% shortening, 1% salt, 3% yeast, and 60% absorption. The same flour was used throughout the baking studies—a commercially milled, unbleached and unsupplemented hard winter wheat flour. The loaves were proofed to a constant height of 9.5 cm. Malt supplements were added to the above formula as desired. In the comparison of different malts a constant increment of 0.1% malt was used throughout. The malts were added as extracts (1 g malt flour plus 100 ml water for one hour at 30°C). Thus a 10-ml portion of malt extract was substituted for 10 ml of water in the formula.

Relationship of Alpha-Amylase to Gassing Power

Since it is more convenient experimentally to supplement the flours with malt extracts than with the malt flours themselves it was necessary to establish the relationship between responses to these two types of addition. Table I shows the results obtained. Two different

TABLE I

COMPARISON BETWEEN THE GASSING POWER INCREASES GIVEN BY MALT EXTRACTS AND EQUIVALENT AMOUNTS OF MALT FLOURS

Malt	Level	Increase in gassing power	
		Flour	Extract
	%	*mm mercury*	*mm mercury*
Wheat	0.1	55	53
Wheat	0.2	68	67
Wheat	0.3	82	80
Barley	0.1	57	52
Barley	0.2	77	76
Barley	0.3	85	90

malts (wheat and barley) were used. They were added as 0.01, 0.02, and 0.03 g of malt flour and as extracts equivalent to these weights. It is apparent that extracts produced essentially the same responses as did flours. Accordingly extracts were used in all the following work.

Two types of extracts were used for studying the relationship of alpha-amylase to gassing power: (1) A wheat malt extract heated at 70°C for 30 minutes to inactivate the beta-amylase (Ohlsson, 1930).

This extract had adequate alpha-amylase activity (41.4 units) and no apparent beta-amylase activity. (2) An extract of a hard wheat flour of established freedom from alpha-amylase. The two extracts may then be designated respectively as an alpha-amylase preparation and a beta-amylase preparation. The extracts were added in increments to both an experimentally milled and a commercially milled hard wheat flour [2] and gassing powers determined. The data are plotted in Figure 1.

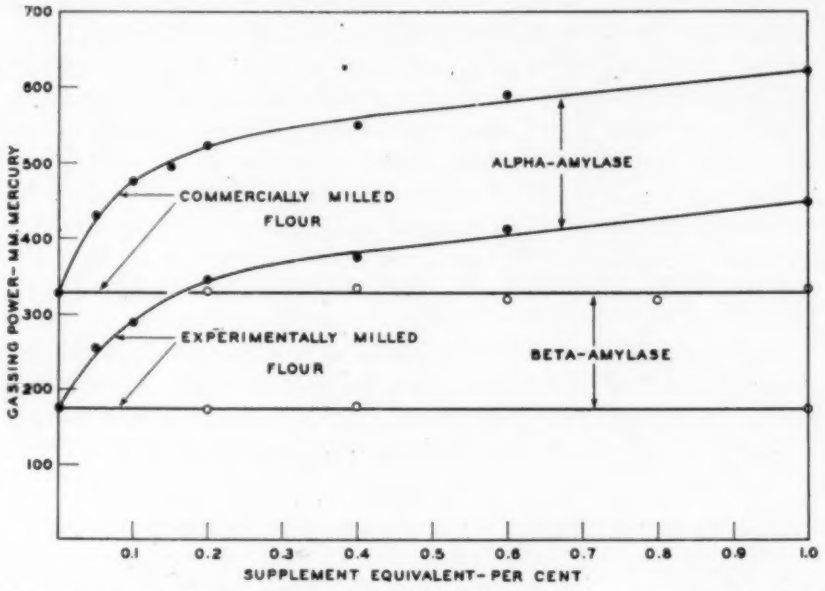

Fig. 1. Relationship of supplementation with alpha- and beta-amylase to the gassing power of flours.

As shown in Figure 1 the experimentally milled flour gave lower gassing-power values than the commercially milled. This would be anticipated on the basis of probable content of available starch. Increments of beta-amylase up to the equivalent of 0.1 g flour gave no increase with either flour. On the other hand as increments of alpha-amylase were added gas production was stimulated greatly with the first small increments and then more slowly at higher levels. Alpha-amylase equivalent to 0.02 g malt was sufficient to raise the total gas production of the experimental flour to a level above that of the unsupplemented commercial flour.

The data of Figure 1 supply experimental proof for the supposition that the enhanced gas production of malt-supplemented, over that of unsupplemented, flours is attributable to alpha-amylase activity.

[2] Neither of these flours was used for the loaf volume and gassing power data obtained with the series of malts and reported under "the evaluation of malts."

Relationship of Alpha-Amylase to Loaf Volume

Three preparations from malt were used as flour supplements in an attempt to isolate the fraction responsible for increase in loaf volume. Unmodified malt extract was used at 0.5% level as a check. This same extract was treated in a manner similar to the Ohlsson (1930) technique for differential inactivation of the two amylase components. Part was heated at 73°C for 30 minutes to provide a beta-amylase-free preparation of alpha-amylase. On analysis it was found that 65.7% of the alpha-amylase activity remained in this extract, while the beta-amylase activity was reduced to zero. The loss of alpha-amylase was greater than is usual with the Ohlsson technique (70°C for 15 minutes) and may be accounted for by the high temperature and prolonged heating used to insure complete inactivation of the beta-component. Another part of the malt extract was acidified to pH 3.3 with hydrochloric acid, held at that pH for 20 minutes at room temperature, then returned to pH 6.0 by addition of sodium hydroxide. This extract retained 77 5% of its beta-amylase activity and only 4.8% of the original alpha-amylase activity.

In the final dilutions of the extracts as prepared for use in baking, the alpha-amylase solution was adjusted so that volume for volume it had the same alpha-amylase content as the untreated malt extract. All three extracts were then used to supplement the baking formula:

TABLE II

RELATIONSHIP OF ALPHA- AND BETA-AMYLASE TO LOAF VOLUME

Malt treatment	Beta-amylase activity	Alpha-amylase activity	Increase in loaf volume	Proof time[1]
	units	*units*	*cc*	*min*
Untreated	8.0	50.0	60	46
Heat treated	0.0	50.0	55	47
Heat treated	0.0	40.0	47	46
Heat treated	0.0	30.0	45	47
Heat treated	0.0	20.0	26	46
Heat treated	0.0	10.0	22	47
Acid treated	12.4	4.8	12	46
Acid treated	6.2	2.4	0	46

[1] "Proof time" is the time required to proof the test loaves to a constant height of 9.5 cm.

the untreated extract at 0.5% level, the heat-treated at levels of 0.5, 0.4, 0.3, 0.2, and 0.1% levels. The baking results are shown in Table II. Alpha- and beta-amylase activities are given, treating each increment as if the same volumes of different malts were being used. The loaf volumes are given as increases over that of the unmalted loaf (653 ml) and are averages of two bakes. In addition the average time required to proof the loaves to a constant height of 9.5 cm is recorded. The data of Table II indicate that increase in

loaf volume was dependent on the alpha-amylase content of the extract used for supplementation, thus supporting the conclusion which Munz and Bailey (1937) reached by a similar approach. The hypothesis that small increments of beta-amylase should not influence loaf volume is supported. Also it is difficult to postulate such recorded increases as due to other enzymic activities typical of malt extracts, since these activities (proteolytic, lipolytic, oxidative, etc.) could not be expected to respond to heat and acid treatments in the same manner and degree as does alpha-amylase.

It is obvious that the rate of fermentation during the proofing of the doughs represented by the data of Table II was not influenced by the kind of diastatic supplement. It must be concluded that the rather wide variation in the volumes of the baked loaves was caused by difference in gas-retaining ability during the baking and *not* by variation in gas production during fermentation.

Evaluation of Malts

Malt supplement is usually added as malt flour. Malt extracts are somewhat more convenient to use experimentally and invariably gave more uniform results in duplicate bakes. Table III shows a comparison of the loaf-volume increases resulting from supplementation by six malts added both as flours and as one-hour extracts, all at 0.1% level. Increases given are the averages of four bakes in each case.

TABLE III

COMPARISON BETWEEN LOAF VOLUME INCREASES GIVEN BY MALT EXTRACTS AND EQUIVALENT AMOUNTS OF MALT FLOUR

Malt no.	Increase in loaf volume	
	Flour	Extract
	cc	*cc*
13	49	44
14	45	43
15	40	38
16	34	24
17	29	26
18	27	18

The average unsupplemented loaf volume was 600 cc. The data indicate that the malt extracts were quite comparable to the malt flours in their influence. Increases resulting from the use of extracts were slightly lower in this case than those given by the flours. The data given in Table III for extracts were obtained several weeks after those for the malt flours so the differences in loaf volume increase between the two methods of supplementation cannot be considered significant.

If both gassing-power and loaf-volume increases from malt supplementation are dependent on the alpha-amylase component there should be a very close correlation between such increases and the alpha-amylase contents of various malts. Accordingly the influence of some 24 malts on gassing power and on loaf volume was determined. Extracts were used in all instances and a constant increment level of 0.1% malt for both gas production and baking response. For convenience the malts were separated into three groups: (A) a series of 12 barley malt flours prepared in the laboratory from corresponding malt grains; (B) a series of six commercially milled malt flours including 4 wheat malts, 1 rye malt, and 1 barley malt; (C) a series of six "wheat malt" meals resulting from six different periods of germination of the same wheat sample. Data are given for saccharogenic activity (both Kneen-Sandstedt units and degrees Lintner), for alpha-amylase activity, for increase in six-hour gassing power, and for increase in loaf volume. In addition, the average proof time is recorded for each supplementation.

TABLE IV

RELATIONSHIP BETWEEN SACCHAROGENIC ACTIVITY, ALPHA-AMYLASE ACTIVITY, AND GASSING POWER AND LOAF VOLUME RESPONSES FOR THE TWELVE BARLEY MALTS OF GROUP A

Malt no.	Saccharogenic activity		Alpha-amylase activity	Increase in gassing power	Increase in loaf volume	Proof time
	Lintner value (calc)	Kneen-Sandstedt value				
	deg	*units*	*units*	*mm Hg*	*cc*	*min*
1	241	32.1	133.7	142	63	49
2	214	28.5	91.5	120	60	51
3	137	18.3	74.5	100	49	50
4	269	35.9	70.5	97	49	50
5	188	25.0	68.7	102	50	50
6	129	17.2	52.2	79	45	51
7	178	23.7	45.8	75	36	49
8	92	12.2	45.0	73	32	50
9	162	21.6	42.8	73	31	49
10	80	10.7	40.1	72	33	50
11	131	17.4	36.2	67	29	50
12	186	24.8	35.3	67	29	49

The data for the 12 barley malts of group A are shown in Table IV. The malts are listed in order of decreasing alpha-amylase activity. Values given for gassing-power increase are averages of duplicate determinations, those for loaf volume increase averages of from 7 to 8 bakes. It is obvious that there is an excellent correlation between increases in loaf volume and increases in gassing power. Equally striking is the high degree of correlation between alpha-amylase

activity and either of these two increases. On the other hand, the saccharogenic (Lintner) activity of the malt bears little relationship to the value of the malt as a flour supplement.

Times required to proof to the constant height were constant throughout the series and, as in all bakes, were essentially identical with the proof time of the unsupplemented loaf.

Table V lists data for the six miscellaneous malts of group B. In addition to the values for amylase activity and increases in loaf volume and in gassing power, the cereal from which the malt was prepared is given. It should be noted that No. 16 is from the same wheat sample as No. 15, its lower activity being the result of a shorter period of germination. Results given are the averages of duplicate gassing-power determinations and quadruplicate bakes. As with the malts of group A (Table IV), there is a high degree of correlation between loaf-volume increase, gassing-power increase, and the alpha-amylase activity of the malt, and no effect of the malt supplement on gas production during the proof. With this group of malts saccharogenic activity likewise shows a fair degree of correlation with dough response. However, No. 17 is so far out of line that here too, as with the malts of group A, the results indicate that little confidence may be placed in predictions of baking response based on saccharogenic values.

TABLE V

RELATIONSHIP BETWEEN SACCHAROGENIC ACTIVITY, ALPHA-AMYLASE ACTIVITY, AND GASSING POWER AND LOAF VOLUME RESPONSES FOR THE MISCELLANEOUS MALTS OF GROUP B

Malt no.	Malt source	Saccharogenic activity		Alpha-amylase activity	Increase in gassing power	Increase in loaf volume	Proof time
		Lintner value (calc)	Kneen-Sandstedt value				
		deg	*units*	*units*	*mm Hg*	*cc*	*min*
13	Barley	155	20.7	65.2	80	44	46
14	Rye	103	13.7	53.5	72	43	45
15	Wheat	68	9.1	31.6	60	38	47
16	Wheat	65	8.6	25.8	47	24	46
17	Wheat	106	14.1	24.3	42	26	46
18	Wheat	47	6.3	22.2	38	18	44

Data on the six germinated wheat samples of group C are given in Table VI. These samples were of a somewhat different nature from usual wheat malts. Sprouts and roots were not removed previous to drying, grinding, and extraction. Sprout length varied from that of No. 24, in which development had just started, to that of No. 19 in which the length was from 30 to 40 mm. The samples are listed in order of decreasing period of germination. Gassing-power and loaf-

TABLE VI

RELATIONSHIP BETWEEN SACCHAROGENIC ACTIVITY, ALPHA-AMYLASE ACTIVITY, AND GASSING POWER AND LOAF VOLUME RESPONSES FOR THE GERMINATED WHEAT SAMPLES OF GROUP C

Malt no.	Saccharogenic activity		Alpha-amylase activity	Increase in gassing power	Increase in loaf volume	Proof time
	Lintner value (calc)	Kneen-Sandstedt value				
	deg	*units*	*units*	*mm Hg*	*cc*	*min*
19	248	33.0	173.9	141	84	50
20	229	30.5	148.9	135	61	49
21	188	25.1	106.4	110	57	50
22	118	15.7	58.0	92	44	51
23	82	10.9	19.3	43	19	51
24	60	8.0	3.5	1	5	51

volume data are the averages of duplicate and triplicate determinations, respectively. As is customary, the malt extraction was for one hour at 30°C and supplementation was at the 0.1% level.

Gassing-power and loaf-volume data, as recorded in Table VI, show excellent correlation with each other and with both alpha-amylase and saccharifying activities. Greater gassing-power and loaf-volume responses were found for No. 19 than for any other of the malts listed in Tables IV to VI. This sample had the highest alpha-amylase content of all. Likewise, the extract of No. 24 was lowest in alpha-amylase content and gave the least response. On the other hand, these samples were not at the extremes when evaluated on the basis of their saccharogenic activity.

The correlation between loaf-volume response and saccharogenic activity, shown by the malts of group C, must be considered as incidental. During the process of germination not only is there an increase in alpha-amylase activity but likewise a liberation of beta-amylase. Saccharifying power, being the result of the combined action of these two components, is therefore dependent on the period of germination. Since other malt enzymes besides the amylases respond similarly to germination, any attempt to evaluate all malts on the basis of a single set, such as that illustrated in Table VI, might well lead to erroneous conclusions.

Correlation Study

Data obtained for the 12 malts in group A, 5 of the 6 malts in group B, and 1 of the 6 malts in group C were analyzed for correlation. Since Nos. 15 and 16 resulted from different periods of germination of the same wheat sample, only data for sample 15, the higher in activity, were included in the analysis. Similarly, only the data for

sample 19, the highest in activity of those in group C, were included. By this means it was insured that any one malt was independent of all others. It has been pointed out above that evaluation based on a series of malts all produced from the same grain sample (*e.g.*, the six germinated wheats of group C) is inherently of little significance.

The correlation coefficients for the group of 18 malts are given in Table VII. Analysis of the data confirms the conclusions arrived at by inspection. When malt supplementation is used as the only variable, increase in loaf volume and increase in gassing power are both dependent on the increment of alpha-amylase added, and within the limits of the experiment may be attributed to this factor. These two increases, therefore, show a high degree of correlation one with

TABLE VII

CORRELATION COEFFICIENTS BETWEEN THE MALT PROPERTIES INVESTIGATED

Malt property	Alpha-amylase activity	Increase in gassing power	Increase in loaf volume
Saccharogenic activity	+.7216	+.7929	+.7126
Alpha-amylase activity	—	+.9383	+.9542
Increase in gassing power	—	—	+.9351

Note: for 16 degrees of freedom the 1% point is equal to +.590.

the other. Saccharifying power is significantly correlated with alpha-amylase activity and therefore with gassing power and loaf volume. This indicates that conditions leading to the development of alpha-amylase activity in germinating grains *usually* influence saccharifying activity in a similar manner. However, no *accurate* prediction of baking response can be made on the basis of malt saccharogenic values either in terms of "degrees Lintner" or any other comparable "unit" of saccharogenesis.

Discussion

The data establish beyond question that alpha-amylase is of significance both in the gassing-power and in the loaf-volume response of flours. This confirms the conclusions of Munz and Bailey (1937), Sandstedt (1938), Sandstedt, Jolitz, and Blish (1939), and Freeman and Ford (1941). It likewise appears that malt evaluation based on the gassing-power technique of Davis and Tremain (1938) is valid, since both gassing-power increase and increase in loaf volume are very highly correlated with the increment of alpha-amylase. If factors in addition to alpha-amylase are influential in the increased loaf volume achieved by malt supplementation, they must be similar to alpha-amylase in their response to acid and heat.

Since both the gassing-power method and the determination of malt alpha-amylase activity are measures of alpha-amylase and thus appear to be equally reliable for evaluating malt, any choice between the two should be based on convenience. Certainly the direct evaluation of alpha-amylase activity is a more accurate and rapid method than indirect evaluation from gassing-power increase. Even assuming that final evaluation is made in the commercial laboratory by determining gassing power or autolytic diastatic response, the malt itself should be purchased on the basis of its alpha-amylase activity. A "high diastatic" or a "strong" malt is not necessarily high in alpha-amylase, the only malt amylase component of any significance for diastating bread flour.

The relationship of alpha-amylase activity to the gassing power of sugar-deficient doughs is readily explained on the basis that the abundance of beta-amylase already present in the flour can saccharify only 60% of the "available" starch of that flour. Alpha-amylase, in combination with the beta component, can carry hydrolysis far beyond that point. In addition, alpha-amylase seemingly is the component of malt responsible for the hydrolysis of "raw," undamaged wheat starch (Kneen, Beckord, and Sandstedt, 1941).

The role of alpha-amylase in baking is obscure. If the sugar level of the formula becomes a limiting factor in fermentation, the sugar produced by the combined action of alpha- and beta-amylase would result in increased gas production and perhaps increased loaf volume. When the sugar level is adjusted, as in the present experiments, in such a manner that gas production is not a limiting factor, loaf volume is dependent upon *gas retention* in the oven. At the present time it appears that one manner in which alpha-amylase action might be effective in gas retention lies in the ability of the enzyme to modify the kind and amount of dextrins in the dough.

Kozmin (1933, a, b) found that in flours of high diastatic activity prepared from sprouted wheat an overabundance of dextrins was produced, this in turn leading to a reduction in bread "quality." Munz and Bailey (1937) noted a pronounced increase in the mobility of doughs supplemented with alpha-amylase and attributed this change to the production of dextrins. Kent-Jones and Amos (1940) found that diastatic supplementation of flour-water pastes markedly increased the "dextrin figure." Freeman and Ford (1941) reported an increase in the dextrin content of bread baked from supplemented flour over that of unsupplemented. Sandstedt, Jolitz, and Blish (1939) concluded that loaf volume stimulation by alpha-amylase was due to its action on the starch and dextrin fractions of doughs. In doughs receiving no malt supplementation there is a considerable

amount of alpha-amylodextrin produced by the action of beta-amylase on "available" starch. Alpha-amylase reduces this dextrin to smaller dextrins and at the same time produces additional dextrins from starch resistant to the attack of the beta-component. Alpha- and beta-amylase acting together rapidly reduce these high molecular weight dextrins to smaller dextrins and sugar. It may well be that at the levels of diastatic supplementation used in the experiments reported above, the increase in gas retention leading to the greater "oven spring" and loaf volume of supplemented flours may be due to such production and *modification* of dextrins.

The intention of this discussion is not to minimize the significance of diastatic supplement in maintaining the gas production of doughs deficient in sugar. Rather it is to point out that "malt response" is a more complex factor than commonly believed. Certainly the data indicate that the ability of a dough to retain gas when baked is influenced by the degree of alpha-amylase activity in the fermenting dough.

Summary

The evaluation of malt for use as a flour supplement has been, and to a minor extent is at present, based on saccharifying power (Lintner value). This saccharogenic activity, though the result of the combined actions of malt alpha- and beta-amylases, is largely determined by content of the beta component. Flour contains an abundance of beta-amylase and consequently could not be expected to respond to the addition of small increments of this component. If amylolytic activity is responsible for malt stimulation of gassing power and of loaf volume, such stimulation must be correlated with the increment of *alpha-amylase* added.

The influence of increments of alpha- and beta-amylase on the six-hour gassing-power values of sugar-deficient doughs was studied. Gas production was not affected by beta-amylase but did respond to additions of the alpha-component. The increase in gas production per increment of alpha-amylase was relatively great for small increments and diminished at higher levels of this component.

Malt extract was treated in such a manner as to give two preparations, one low in alpha- and high in beta-amylase, the other high in alpha- and deficient in beta-amylase. The baking responses of these were compared with that of the original extract. Even though the method of baking eliminated gas production as a variable, in all instances loaf-volume response was proportional to the increment of alpha-amylase added.

Extracts from 24 malts were used to supplement flours and resulting increases in gassing power and loaf volume determined. Both

of these responses were very highly correlated with the alpha-amylase activity of the extract and therefore with each other. Correlation between loaf volume and saccharogenic activity of the malt was significant but could be considered only as incidental and dependent on the tendency for malts of high alpha-amylase activity also to have high saccharogenic activity.

Throughout the wide range of alpha-amylase supplement used in the experimental bakes the times required to proof to constant height showed no significant variation. This demonstrated that malt response under such conditions was *not* dependent on modification of *gas production*.

The data indicate that consideration of alpha-amylase activity is of major importance in evaluating diastatic supplements of flour. Accordingly the purchase of malt supplement should be governed by the content of alpha-amylase, not by the ability of that malt to perform some unrelated function.

It is pointed out that the role of alpha-amylase in modifying the volume of test loaves is complex. In sugar-deficient doughs the role of malt supplement in maintaining gas production is obvious. The present study under conditions in which gas production was not a variable illustrates another manner in which alpha-amylase supplementation influences loaf volume: by modifying the *gas-retaining* capability of the dough during baking. The probable effect of this enzyme on the quantity and quality of the dextrins in dough is discussed.

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A MICROSCOPIC STUDY OF BREAD AND DOUGH

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The microscopic components of bread are well known but their physical relationships have usually been surmised by indirect means. This direct microscopic study of bread and its dough entails (1) a review of microscopic structural studies, (2) the devisal of a practical sectioning technique that was used on (3) a series of samples taken from bread, both during and after its preparation, (4) a microscopic examination of the structures and their changes, and (5) some theoretical inferences based upon the foregoing. This report presents the progressive changes during breadmaking, as well as the minute structures themselves.

Many microscopic studies of breadstuffs are concerned with the individual bread components, such as yeast, starch, or other identifiable substances. However, only a few studies have pictured or described the physical relationships of the components. One of the earlier ones was the work of Scheffer (1916). This was illustrated with photomicrographs. Another early study was the article by Verschaffelt and van Teutem (1915). This work has been referred to and reproduced by J. R. Katz in several subsequent articles. Their illustrations are camera lucida drawings from sections of bread. They described the gluten structure in which starch and yeast are embedded, elongation of the starch grains adjacent to air cells, lines of flow similar to those seen on a cut steel surface, and some other phenomena which were attributed to staling changes. A third structural study was made in 1930–31 by Joseph Stagman, Chicago, Illinois. This has not appeared in the literature. He worked with white, rye, and wholewheat breads. The sections were made from washed gluten, sponge, dough, and the bread. In addition to the gluten framework for starch and yeast, he describes the gas pockets and their enlargement during fermentation and baking. Butterworth and Colbeck (1938) published several photomicrographs made from both bread and dough preparations. They described the effect of stretching upon sponge and dough, a "squeezing-out" of the starch from the gluten mechanically produced by leavening, as well as the gluten meshwork in which are embedded the yeast and starch. Each of these studies centers around the

methods used in producing the sections. However, they all agree as to the function of the gluten.[1]

Sectioning Technique

Microscopic preparations for showing progressive changes must disclose representative cross sections of the original specimens. The technical difficulties are twofold, thinness for clarity and the prevention of visible changes. The latter is especially troublesome in breadstuffs because there are no cell walls, skeleton, or analogous structures as in the usual histological material. The gluten acts as a framework, but since its several fractions are soluble in various solvents, the common ones cannot be used. Slight mechanical manipulation inordinately changes the microscopic picture, while processes incidental to preparation often cause physical and chemical changes of a fundamental nature. Thus alcohol, water, ether, and xylol cannot be used; teased material, films, and smears show extensive deviations from the original structure; fermentation and enzymatic action must be completely inhibited; and even the effect of temperature and moisture must be fully considered.

The second difficulty lies in making the preparations thin enough to give a clear microscopic picture. The higher the magnification the thinner these must be made. Those which are too thick appear on the photographic plate as a confused mass in which little can be discerned. For magnifications of 500× or more, the preparations should be 10 microns or less in thickness. Besides those previously mentioned, frozen sections are impractical because of their thickness. Embedded specimens can be cut into thin sections which are adequate. These and other considerations prevent the use of many standard procedures and make the problem of true structural representation a difficult one.

The method of producing the accurate cross sections of bread and dough described herein will be detailed later in a separate article. The highlights of this method are the use of CO_2 ice to stop fermentation, enzyme action, and aging; simultaneous *in toto* fixation and staining by osmic acid vapor; dehydration by CaO; embedding with paraffin; cutting on a sliding microtome; albumen fixation of the sections to the slide; staining as desired; and finally mounting in balsam. Samples of sponge, dough, bread, and the ingredients are all successfully sectioned by this method. The resulting sections can be made

[1] During the preparation of this report two other articles have appeared describing pertinent facts and conclusions. Baker (1941), working with highly oxidized doughs, prepared photographs of films of excessively proofed bread. His conclusion, confirmed by the failure of iodine to stain the surface of the gas pockets, was that there is a glutinous lining of these pockets. Baker and Mize (1946), utilizing macroscopic methods, conclude that the entrained gases, the yeast organisms, and manipulations such as punching and molding are of no consequence as the origin of the gas cells. It further concludes that the gas cells arise in the gluten. Material to be presented in this article corroborates these conclusions by visual evidence.

8 microns or less in thickness and stained with specific stains so that the fats and fatty acids are black and the starch is blue. A variety of differential stains distinguish the acidophilic gluten proteins from the basophilic yeast cells. The combination of red yeast cells and yellow gluten produces a striking picture with the blue starch and black fats.

This technique was applied to a progressive series of samples from a simple white dough. For simplicity, a straight white dough of the following composition was used:

Flour	100%	Corn sugar	4%
Water	62%	Salt	2%
Powdered skim milk	4%	Yeast	2½%
Lard	4%	Malt	½%

The dough was mixed 7 minutes at 56 rpm, scaled and rounded immediately, allowed 30 minutes on the bench, made up by hand, proofed at 90°F for about 55 minutes, and baked 28 minutes in a brick peel oven. The dough schedule and corresponding samples were as follows:

Schedule (min)	Sample	Sample No.
	Ingredients	As marked
0	Just out of mixer	1
10	Rounded, 10 min on bench	2
30	Rounded, 30 min on bench	3
30	Immediately ff makeup	4
40	10 min in proof box	5
85	To oven ff 55 min proof	6
160	Bread after cooling	7

The presentation of sections begins with the ingredients and follows in the order of the samples as listed.

Microscopic Findings

The microscope was a Carl Zeiss medical-type instrument. A 8× ocular was used with 3×, 8×, 40×, and 100× oil-immersion objectives. The camera was a 35-mm focal-plane shutter type. The magnifications given below are purely relative. This stain was a combination of carbol-fuchsin and Bismark Brown, unless otherwise stated.

The flour used in the preparation of the dough was a short-patent hard-wheat commercial variety. Figure 1 indicates the nature of flour granularity. The particles vary in size from individual starch grains to a group of 20 or more cemented together by a matrix of gluten. Since this is a cross section, the individual particles of flour have more starch grains than can be seen in the pictures. Likewise, the average diameter of the flour particles is larger than is apparent,

because the knife of the microtome is more likely to cut through a smaller portion than at the widest part. The large particle of flour in Figure 2 shows no systematic arrangement of starch. Figure 3 brings out the typical structure of wheat starch; the small grains are nearly spherical; the intermediate ones, ovoid; and the large ones,

FLOUR

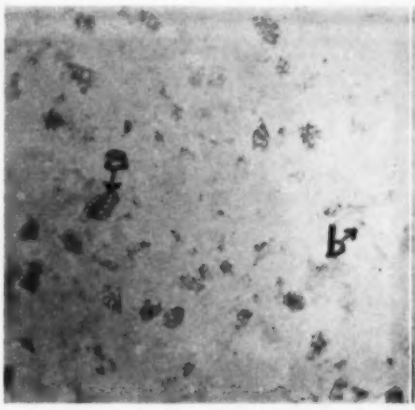


Fig. 1. Flour: *a*, large particle; *b*, small particle. 64 ×.

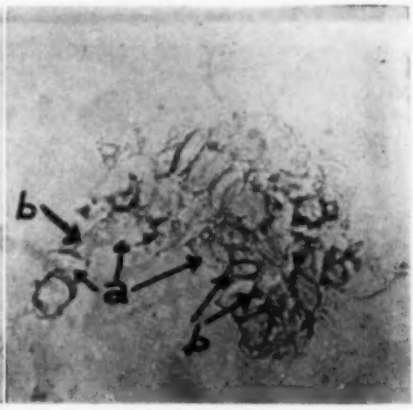


Fig. 2. Flour particle: *a*, starch; *b*, gluten matrix. 320 ×.

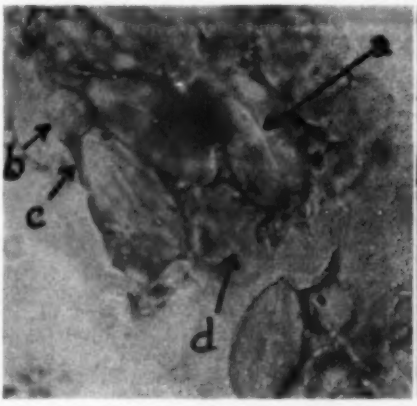


Fig. 3. Flour: *a*, edge of large starch grain; *b*, small spherical grain, on surface; *c*, gluten on surface; *d*, hilum of grain. 800 ×.

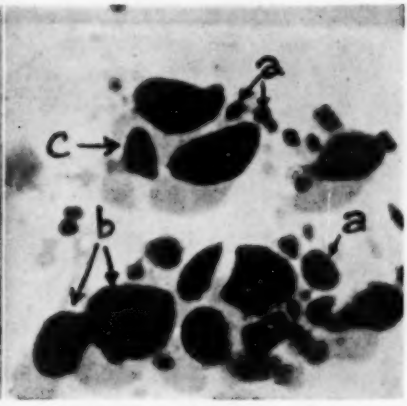


Fig. 4. Flour: (deep iodine stain) *a*, small starch grain; *b*, large starch grain; *c*, broken grain. 800 ×.

either elliptical with the median "crease" (*i.e.*, edge), or roughly ovoid, depending upon the aspect presented. The starch lies in contact with the gluten matrix, yet well defined from it by a smooth, uniform, fine line. Figure 4 was deeply stained with iodine (Lugol's solution) causing the starch to stain an opaque blue-black. The starch grains vary widely in size and shape; two broken ones are shown.

Powdered skim milk (Fig. 5), roller process type, shows a longitudinal cross section through a large flake. Figure 6 shows the same, except that it has been cut across the flake. Figure 7 is a higher magnification of Figure 5. Figure 8 shows an unstained flake. Powdered milk cannot be identified when mixed into dough as it has no characteristically formed elements and no staining reaction.

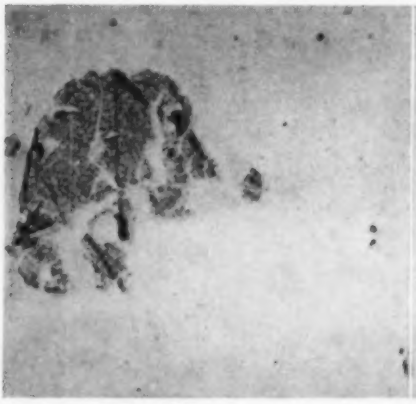


Fig. 5. Milk flake. 64×.

Fig. 6. Milk, cut across the flakes. 64×.

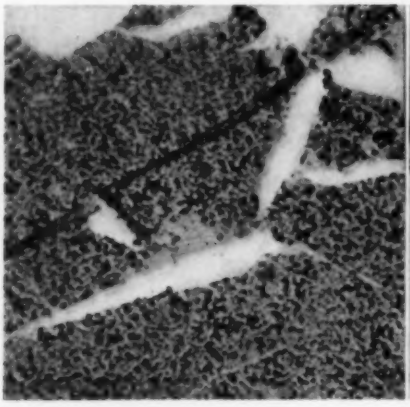


Fig. 7. Milk flake. 320×.

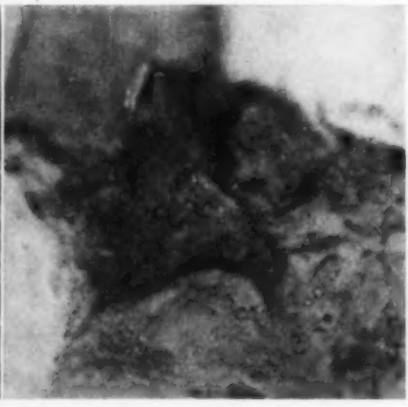


Fig. 8. Milk, unstained. 800×.

Lard was run through the same preparatory technique as the other samples and Figures 9–11 were obtained. In relatively large amounts it does not show formed elements nor its characteristic staining reaction, and consequently has an entirely different appearance in breadstuffs.

The first dough sample was taken directly from the mixer. Figure 12 shows a confused mass of starch and the darker gluten. There are

no gas bubbles present and no order or alignment of the starch and gluten is apparent. Figure 13 shows an enlarged view of the same and brings out the lack of organized structure. In Figures 13 and 14, and with the other high magnifications of dough, small irregular spaces are seen which are artifacts resulting from the shrinking of the gluten away from the starch, due to dehydration during preparation. Both figures also show, evenly dispersed in the gluten matrix, small, dark,

LARD

Fig. 9. Lard. 64×.

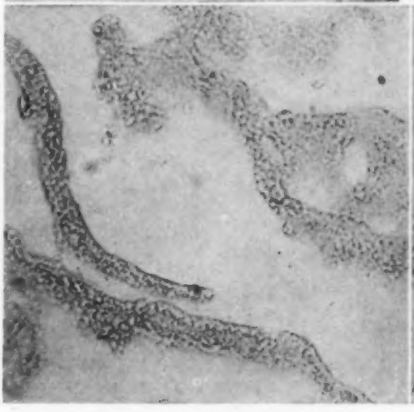


Fig. 10. Lard. 320×.

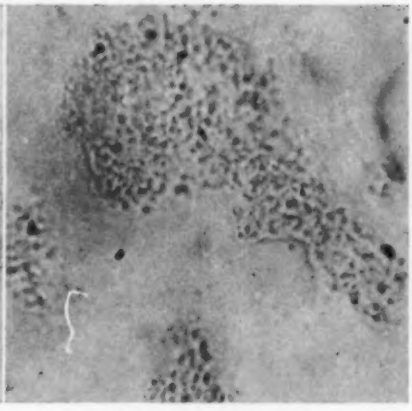


Fig. 11. Lard. 800×.

oval areas, which are deeply stained yeast cells. Fat stains a deep brown or jet black, but throughout the sections of dough there are no black areas which approach even the size of the yeast cells. But, wherever the edge of starch granules may be clearly discerned, there is often a black beading along the periphery, as may be seen in Figure 14. Since these are not seen in flour (Fig. 3) and the black stain is a specific stain for fat, this may be the dispersed shortening.

The second sample of dough was taken 10 minutes after mixing. Figure 15 shows two small gas bubbles which are the beginnings of leavening as produced by yeast fermentation. Some concentric axial alignment of the starch and gluten may be seen just peripheral to each bubble, while the intervening dough shows none. Figure 16 is an enlarged view adjacent to a bubble. This picture shows the even

DOUGH—SAMPLE 1

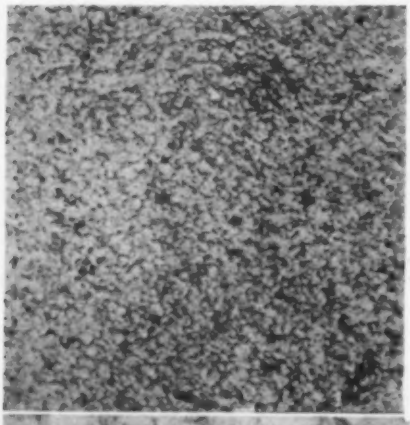


Fig. 12. Dough. 64×.

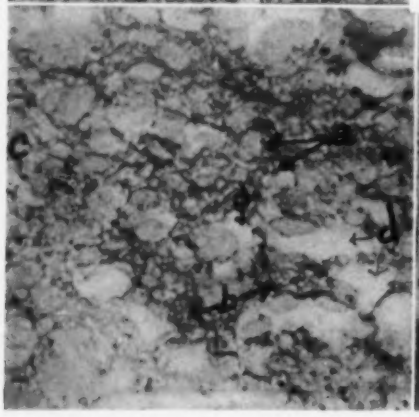


Fig. 13. Dough: *a*, yeast cells; *b*, gluten; *c*, starch; *d*, spaces (artifacts). 320×.

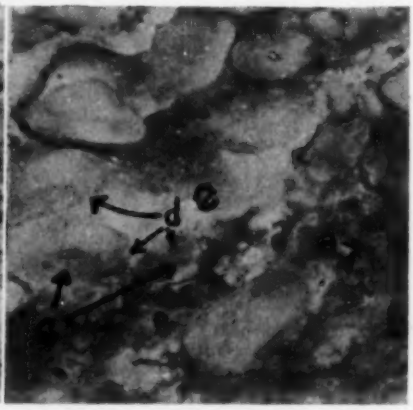


Fig. 14. Dough: *a*, yeast cells; *b*, gluten; *c*, starch; *d*, black beading; *e*, spaces (artifacts). 800×.

dispersion of the yeast bearing no relation to the bubble. Figure 17 is an enlarged view of Figure 16 showing a very small sector of the edge of a gas bubble. As can be seen, both gluten and starch form its walls. The matrix conception of gluten is quite evident, showing large and small starch granules as well as yeast, dispersed in a gluten background. Again the black dots on the periphery of the starch granules are evident.

Sample 3 had 30 minutes on the bench, being ready for makeup. The principal way in which Figure 18 differs from the corresponding Figure 15 (sample 2) is the presence of more and enlarged gas bubbles. Figure 19 shows both gluten and starch forming the gas bubble wall. Figure 20 shows black beading on surfaces of gluten where it has shrunk away from the starch as well as on the starch. The findings are otherwise the same as before.

SAMPLE 2

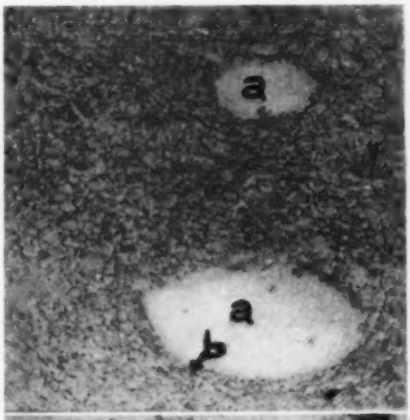


Fig. 15. Dough: *a*, gas bubbles; *b*, slight concentric changes. 64 ×.

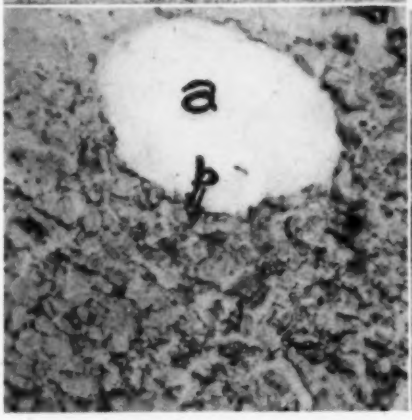


Fig. 16. Dough: *a*, small gas bubble; *b*, yeast. 320 ×.

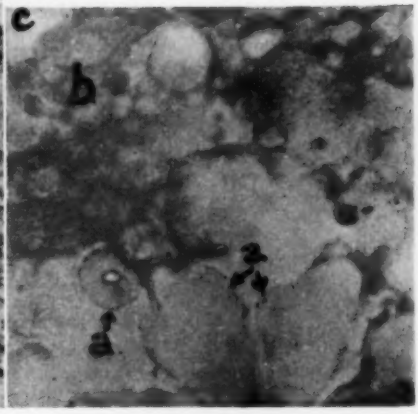


Fig. 17. Dough: *a*, typical peripheral beading; *b*, gluten matrix; *c*, gas bubble. 800 ×.

Sample 4 was taken after the dough was made up by hand. Figures 21 and 22 demonstrate alignment of the starch granules and gluten other than concentrically around the gas bubbles. There are many groups of several large starch granules which are seemingly arranged in layers. The gluten appears as strands which send off interlacing fibrils to separate the starch. The gas bubbles are deformed and fewer, though not absent, as may be accounted for by the hand makeup

which does not expell all the gas. Figure 23 demonstrates that the gluten is not actually in strands and fibrils, but that there is segregation of gluten and starch. The larger grains of starch are segregated much more than the smaller, which, as well as the yeast, remain embedded at random in the aggregated gluten. Figure 24 (high magnification) shows nothing not previously demonstrated, but it does show

SAMPLE 3

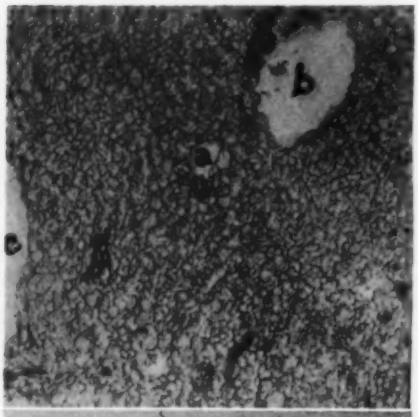


Fig. 18. Dough: *a*, small gas bubbles; *b*, medium gas bubbles; *c*, large gas bubbles. 64×.

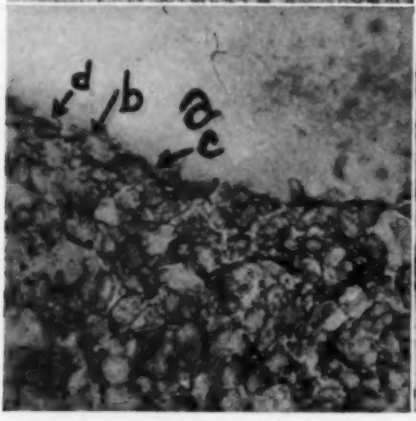


Fig. 19. Dough: *a*, gas bubble; *b*, alignment of starch; *c*, gluten portion of bubble wall; *d*, starch portion of bubble wall. 320×.

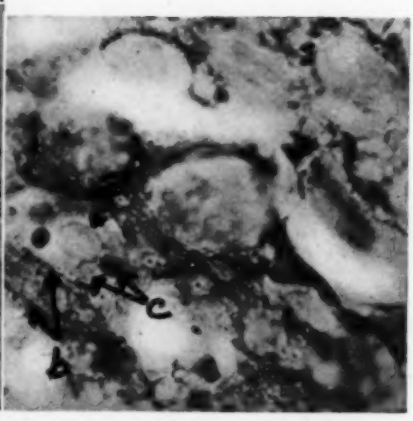


Fig. 20. Dough: *a*, peripheral beading; *b*, beading on shrunken gluten surface; *c*, comparative sizes of yeast and small grains of starch. 800×.

the presence of numerous specks of dust. It is significant that the above changes appear after the process of makeup.

The dough for Sample 5 was taken after 10 minutes of proofing, following the hand makeup. The three figures show no changes other than more gas bubbles and their enlargement. Figure 25 shows rather well (at "*c*") stratification and aggregation of the starch and gluten.

Figure 26 also illustrates this and clearly presents the wall of a gas bubble. This picture is typical of all of the walls of the gas bubbles throughout the dough samples. Both starch and gluten form the surface of the wall. There is the characteristic concentric alignment of the starch axes, while together the starch and gluten form the con-

SAMPLE 4

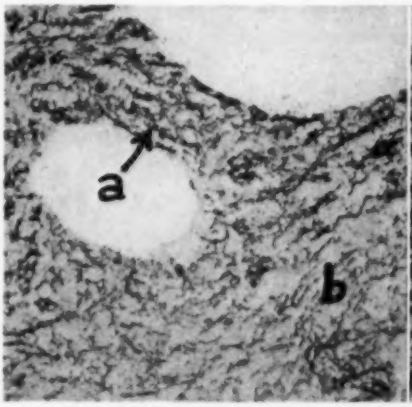


Fig. 21. Dough, lightly stained: *a*, concentric alignment; *b*, stratification and grouping. 64 ×.

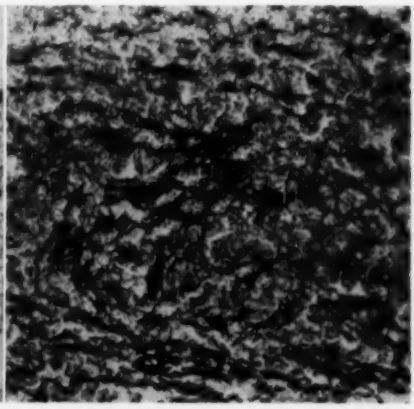


Fig. 22. Dough, gluten deeply stained: *a*, gluten stratification (gluten "strands"); *b*, groups of starch. 64 ×.

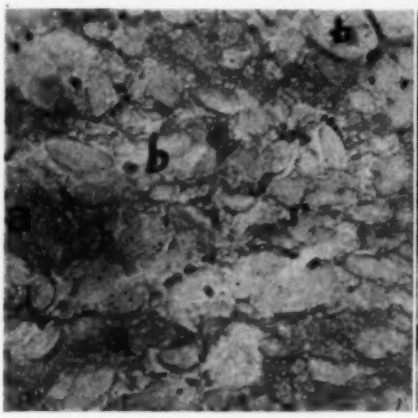


Fig. 23. Dough: *a*, aggregated gluten; *b*, groups of starch. 320 ×.

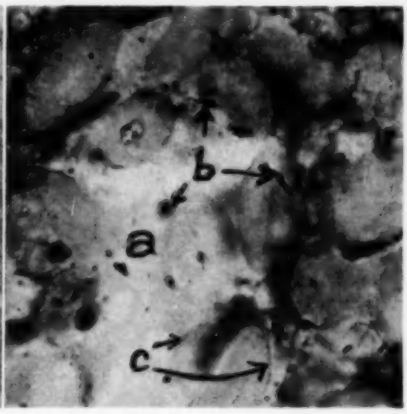


Fig. 24. Dough: *a*, shrinkage space; *b*, dust artifacts; *c*, beading. 800 ×.

centric strata, as previously mentioned under Sample 2. In Figure 27 some of the starch granules show distinct peripheral beading, indicating fat; the yeast shows particularly well, while the gluten appears somewhat granular.

Sample 6 was taken 55 minutes after being made up, at which time the loaves were judged ready for the oven. Figure 29 (also Fig. 28, under Sample 7) demonstrates that the structure is changed from a more or less solid dough mass with an occasional gas bubble to a porous one of numerous large and small gas pockets with walls of intervening dough (the "air cells" of the bakers). The gluten and

SAMPLE 5

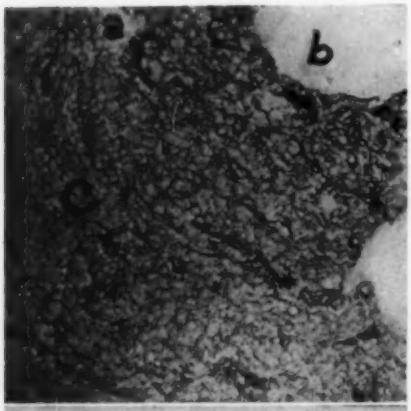


Fig. 25. Dough: *a*, early bubble; *b*, bubbles; *c*, intervening dough structure. 64 ×.

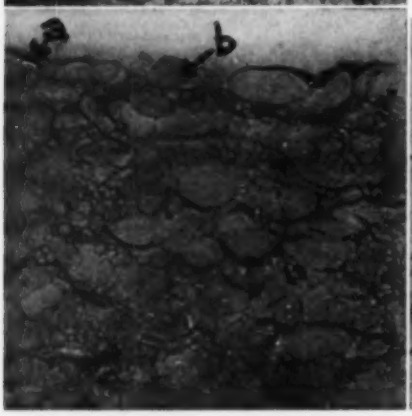


Fig. 26. Dough: *a*, gluten lining of bubble; *b*, starch wall. Note horizontal alignment of starch axes and "layers" of gluten. 320 ×.

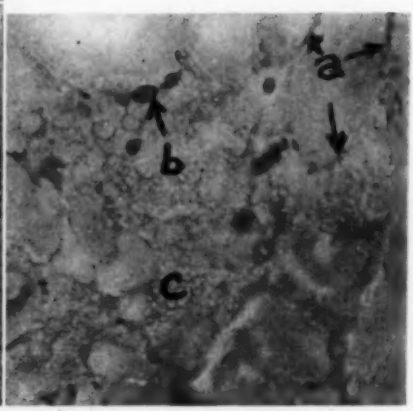


Fig. 27. Dough: *a*, beading; *b*, large yeast cell; *c*, gluten. 800 ×.

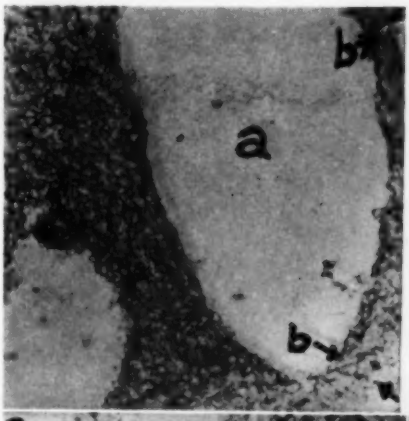
starch arrangement shows that the concentric alignment characterizes a much greater part of the dough structure at this stage. Figure 30 was taken at the junction of three gas pocket walls and shows no deviations of structure. Figure 31 shows no changes from previous samples.

The specimens for Sample 7 were taken from the cooled bread crumb. The gas cells are so large and numerous that their comparison

before and after baking is possible only with low magnification. Comparison of Figures 28 and 32 (dough just before baking and bread just after, respectively) indicates that baking causes enlargement of the gas cells, an increase in their number, marked thinning of the cell walls, and a sharp increase in the number of intramural gas pockets.

Figure 33 is bread under the same magnification as the low powers for previous samples. The moderate-sized gas cells and intramural

SAMPLE 6



(Fig. 28 is on page 208.)

Fig. 29. Dough: *a*, moderate size gas bubble; *b*, thin "cell" walls. 64×.

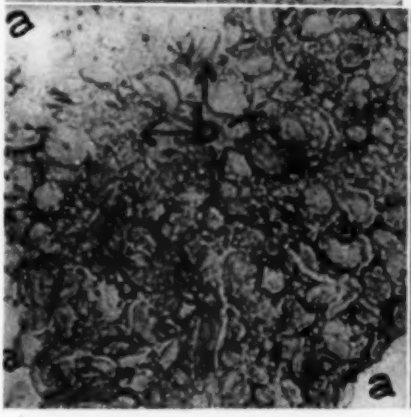


Fig. 30. Dough (poor focus): *a*, gas cells; *b*, random yeast dispersion. 320×.

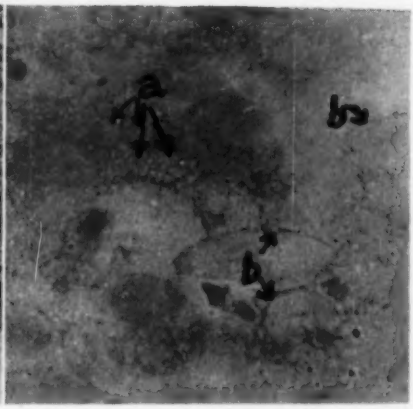


Fig. 31. Dough: *a*, small embedded starch grain; *b*, peripheral beading. 800×.

pockets are shown. At "*c*" is an area which represents a longitudinal section of wall between two gas cells, hence tangential to them. The starch is nearly transparent, while the deeply stained gluten and yeast (the fine black dots) outline it. The concentric stratifications remain around the gas pockets, while the intervening walls show that these extend outwards to merge imperceptibly with the next or confluently change direction in the tangential area to correspond to each other.

Figure 34 shows the junction of a very thin and a moderately thin wall. In the thinner one, starch is the major structure, the gluten being extremely thin or perhaps even absent. The starch at "*a*" shows extreme elongation while at "*c*" very little change is apparent.

BREAD—SAMPLE 7

Fig. 28. Dough (Sample 6): *a*, large gas cell; *b*, medium gas cell; *c*, intramural gas pocket; *d*, cross section of typical cell wall. 24×.

Fig. 32. Bread (Sample 7): *a*, gas cells; *b*, intramural gas pockets; *c*, cross section of cell walls. 24×.

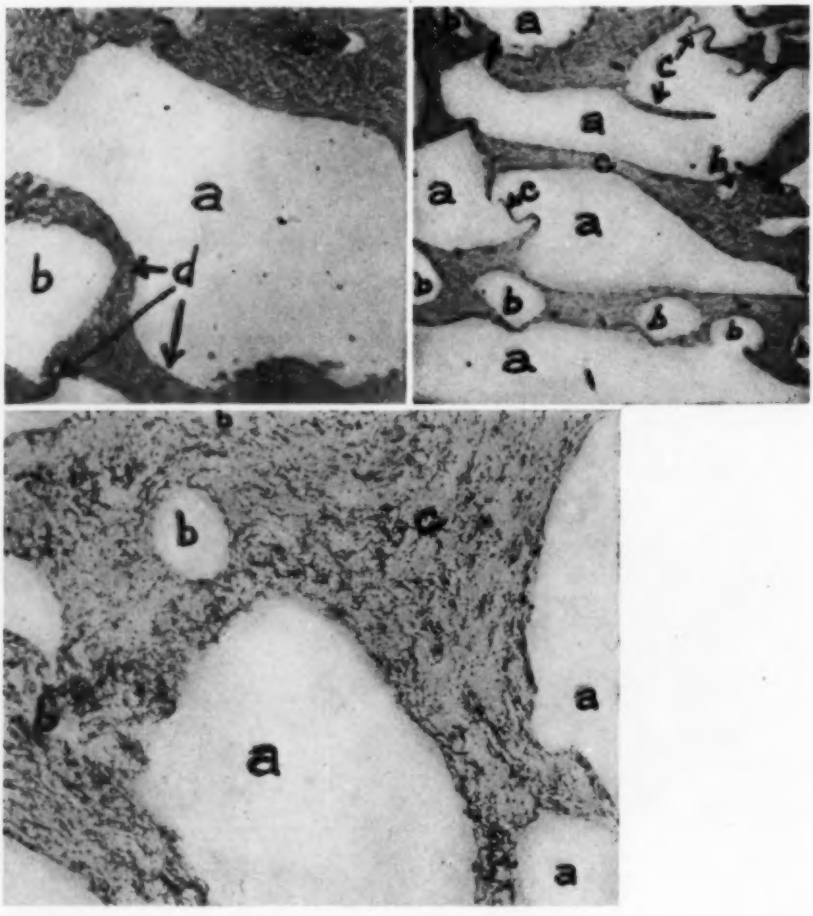


Fig. 33. Bread: *a*, medium sized gas cells; *b*, intramural gas pockets; *c*, tangential cell wall. 64×.

Figure 35 shows one of the thicker cell walls. At "*a*" there is a group of swollen but individual grains of starch with no material between them. Many of the starch grains (as at "*c*") appear somewhat swollen but otherwise unchanged, even retaining the faint crease of the

edge. At "*b*" and other places are black areas of irregular shape, varying in size up to that of a small starch grain. These black areas demonstrate the specific staining reaction for fats. The apparently unchanged yeast cells remain at random in the gluten matrix. Both Figures 34 and 35 show that the gas-retaining walls are surfaced with gluten and starch.

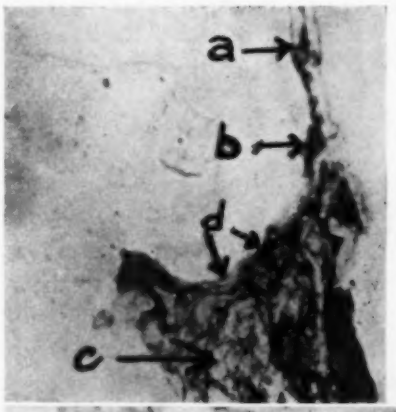


Fig. 34. Bread, thin cell wall: *a*, "stretched" starch; *b*, gluten of thin wall; *c*, "static" starch; *d*, starch and gluten surface. 320×.

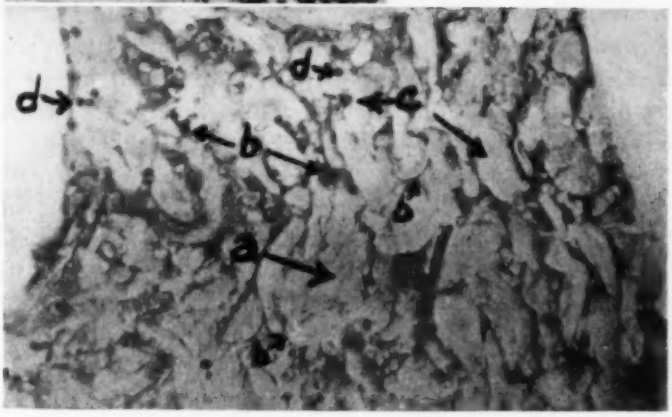


Fig. 35. Moderate cell wall: *a*, swollen starch; *b*, specifically stained fat; *c*, apparently unchanged starch; *d*, yeast cells. 320×.

Figure 36 is a picture of a typical moderately thin cell wall. The fat, as seen at "*b*" and other places, occurs in variously sized droplets from very small globules to irregular masses several times the area of a yeast cell. The larger lipoid masses are in contact with the starch while the smaller globules may also occur in the gluten. This picture is otherwise an enlarged view of the structural features presented under Figures 34 and 35.

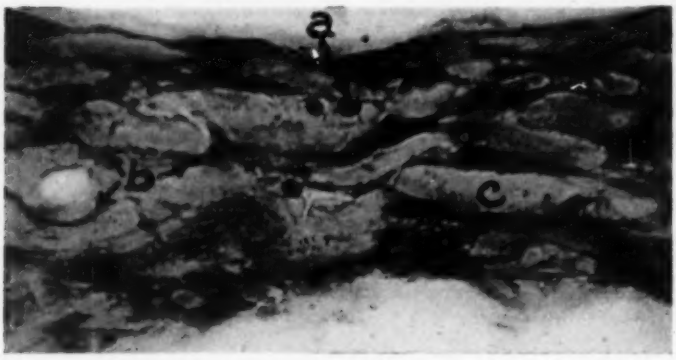


Fig. 36. Bread, cell wall: *a*, yeast cell showing nucleus; *b*, specifically stained fat; *c*, starch; *d*, gluten. 800×.

Discussion

The foregoing illustrations were made sequentially to show, where possible, the structural changes in the material during its manufacture. Causes and effects are discussed under the headings (1) effects of mixing, (2) effects of processing, and (3) changes during baking. While osmic acid was used primarily as a fixative, it coincidentally and selectively stains the lipoid substances. This feature makes possible a study of (4) the role of shortening. Immediate freezing of the samples and their fixation without thawing accurately preserves the microscopic features and consequently enables a logical hypothesis of (5) the mechanism of yeast leavening and the rational (6) correlation of microscopic structures with crumb quality. These and other preliminary studies did not reveal changes in the dough substance directly ascribable to fermentation nor show changes in bread attributable to staling. This does not mean that such are absent, but that further critical studies are indicated.

Effect of mixing: Comparison of the flour and first dough sections (Figs. 1–4, 12–14) demonstrates that during mixing the particles of flour lose their individuality. Furthermore, the even dispersion of the yeast indicates the breaking down and *complete* dissolution of these particles. The total absence of obvious lipoid masses in the dough shows that the shortening is colloidally dispersed at this early stage. In summation, mixing produces a homogeneous suspension of starch, fats, and yeast in the glutinous medium constituting the dough.

Effect of processing: The sequence of dough samples illustrates changes referable to the intermediate proof, molding and the prebaking proof. As anticipated, generalized alterations are not microscopically demonstrable as a result of cutting, scaling, or rounding. Gas pro-

duction first becomes apparent in the second dough sample (Figs. 15–16). As a result, tension occurs in the matrix just peripherally to each gas pocket and produces the tangential alignment of the long axes of the starch. This has been described by Katz and others and apparently always accompanies gas production. Sample 3 indicates further formation of gas by an increased number of variously sized pockets as well as by simple enlargement.

That molding (Sample 4) knocks out much of the gas is evidenced by reduced number and by irregularity of the spaces. The arrangement of the starch is not lost but is extended generally throughout the mass by the coincidental stresses longitudinal to the resulting sheet of dough. Through the "squeezing-out" mechanism of Butterworth and Colbeck (1938), the starch grains tend to segregate into groups of about 10 to 15 each. These groups are made predominantly of the larger grains and evidently are an important consequence of molding (*i.e.*, "mechanical development"). This conversely coalesces the remaining matrix into sheets which appear in section like strands (Figs. 21–22) entwined between the starch. The known improvement in the dough and the overlapping of these sheets are direct evidence of the resulting better gas retention. Following molding there ensues a prolonged period of leavening in which the mechanisms remain the same but the picture becomes remarkably altered. At this time gas pockets reappear in increased numbers and enlarge far beyond previous limits. The impression changes from one of entrapped bubbles in the dough to the porous, honeycomb structure of Sample 6. The apparent strands of the intervening walls (Samples 5 and 6) become confluent. The walls and the gluten are obviously thinned by extension. There is little evidence indicating that rupture is the principal means of bubble enlargement.

Changes during baking: After dough fermentation, the action of heat in baking soon produces crust formation and a rather sudden volume increase, while later the entire mass becomes relatively dry and rigid. Underlying these gross changes are the alterations of the minute structures, including the starch, shortening, gluten, and gas pockets. The ovenspring results from three factors: first, faster gas production as discussed separately; second, further extension subsequent to both the softening of gluten by heat and plasticization of the starch; and third, the rapid appearance of numerous new bubbles in the walls of the preexisting ones (Figs. 28 and 32). Increased extensibility is evidenced by the remarkable thinness of the bubble walls and of the gluten "strands." Plasticity of the starch is shown by its conformity and elongation as part of the walls. Since this is a stage of gelatinization, it is coincidental with the dehydration and fixation of the gluten. Figures 33, 34,

and 35 attest that wheat starch neither ruptures nor coalesces while gelatinizing the outlines of individual grains, their "creases" and freedom from spherical masses demonstrate this. The altered distribution of the lipids indicates that coalescence takes place as described later. Visual changes are not shown in the coagulated protein substances, *i.e.* the gluten and yeast.

The role of shortening: Frey and Landis (1932) express the commonly held view and cite literature to the effect that shortening has lubricating action in the dough and is related to surface tension in some poorly understood fashion. The presented series indicate that not only are both probable, but that both are expressions of the same underlying phenomenon. The lipids of dough are derived from the flour and from the ingredients added at mixing, notably the shortening. Flour extractions yield 1.3% or more fatty substances, varying with the flour fraction, and based on methods which are known to only partially extract the lipids (Sullivan, 1940). The microscopic examination (not shown in the pictures) of flour reveals occasional minute black deposits of stained lipids lying in the protein matrix. These are smaller than the smallest starch grains and may or may not lie in contact with them. The picture becomes very different in the dough samples. The pictures from Samples 1 to 6 show the absence of the black deposits, frequent spaces between the starch and gluten, and a fine black beading at the edge of the starch grain, or on the edge of the shrunken gluten. The absence of stained fat deposits appears significant because more lard (4%) than yeast (2.5%) was added to the flour, yet the yeast is easily identified as oval bodies 5 to 7 microns in diameter, while the lipids are not, despite their specific staining reaction. The possibility of a faulty technique in dissolving out the fat is contraindicated by the presence of the staining reaction in both flour and bread, and by the sample of lard which was taken through precisely the same preparation.

One can conclude, then, that the dispersion of the lipids in dough is a very fine one, perhaps bordering upon the ultramicroscopic. The last two findings (*i.e.*, spaces and beading) are presumptive evidence that the shortening is spread on the interfaces between the relatively dry surface of the crystalline-like starch grains and the moist gluten. The beading is direct visual evidence while the spaces indicate that something interferes with the adhesion of the gluten and starch. Furthermore, the fineness of dispersion on the starch surface is the natural consequence if the 0.04 g of lard per gram of flour (as used in formula) even partially covers the 2,004 sq cm of starch surface as calculated by Stamberg (1939).

The indirect and negative evidence also suggest that the shortening of the dough is dispersed upon the starch surface. The impenetrable

nature of both the yeast cells and starch grains renders unlikely any dispersion within their substance. The gluten matrix is a moisture-bearing colloidal system that has little affinity for hydrophobic substances such as lard. Moreover, the bread sections do not fulfill the anticipated picture that would result from a suspension of fats in the gluten matrix. In such a case, the bread and dough sections might manifest deposits of lipids within the gluten; if the actual dough sections represent a fine gluten dispersion, the bread sections should retain the beading along the starch, the spaces between the starch and gluten, and should not reveal the obvious collections of lipids in contact with the gelatinized starch.

In contrast, the presented sections better fulfill the original premise. During baking, the fat coalesces into larger droplets that bear relation to the starch because of the previous surface dispersion. This coalescence is a rational consequence of gelatinization which, by water absorption, alters the capillary forces acting on the fats. The running together of the fats removes the interposing material between the gluten and starch. The resulting adhesiveness is shown in the pictures by the absence of the starch-gluten spaces. Finally, these considerations rationally correlate the large, specifically stained, lipoid deposits that appear in the bread sections, the other microscopic observations, and the physico-chemical properties of the components. Briefly, the shortening is dispersed upon the interfaces of the starch and gluten within the dough, but coalesces into droplets during baking.

Mechanism of leavening: Yeast contains, among other enzymes, intracellular zymase which breaks down glucose to CO_2 and alcohol.

As stated, this action is intracellular and not diffused through the dough, so one anticipates gas bubble formation to bear some direct relationship to the position of yeast cells. However, the microscopic findings show that practically all of the yeast is embedded, at random, deep within the gluten matrix.

The CO_2 formed within the yeast cell diffuses through the cell membrane into the surrounding moisture-containing suspension, where at first it remains in solution. In physico-dynamic terminology, the cohesiveness of the gluten is greater than the vapor pressure of the CO_2 in solution. By the laws of diffusion the CO_2 pressure tends to equalize itself, but since the CO_2 is continuously produced, there is a decreasing pressure gradient radially from each yeast cell. This gradient is more complicated than in a simple suspension because the starch contains only a little "bound" water, and consequently the CO_2 diffuses around these granules. However, where several of these gradients meet there forms an area of higher vapor pressure which lies between the several yeast cells, but, in cross section, bears no apparent

relationship to them. When the vapor pressure exceeds the cohesiveness, the gluten gives way at its weakest point within the high pressure area. Mechanically a group of starch granules with little intervening gluten forms a weak point and simultaneously serves as a nucleus for gas formation. As the gluten gives way, gas pockets form. Continued CO_2 production enlarges existing bubbles by diffusion and the resulting tension raises the vapor pressure to form others.

During baking the mechanism becomes complicated by the several effects of temperature. Its progressive rise renders the CO_2 less and less soluble, causes its expansion, and so affords continuous leavening action through most of the baking process. Initially the rising temperature also stimulates zymase action so that at 50°–55°C (beginning gelatinization of wheat starch—Herter, 1921) there is heightened gas production. The increased extensibility, resulting from plasticization of the starch and softening of the gluten, and the heightened gas production coordinate to rapidly distend existing gas cells and produce numerous new ones in the matrix. Simultaneous lipid changes favor gluten-starch adherence and consequentially facilitate gas retention thereafter. The interplay of these factors results in the gross effect of "oven spring." Further rise in temperature slows and finally inactivates zymase at about 65°C (Frey and Landis, 1932), but coagulation of the gluten is probably well under way (Alsberg, 1928). The still higher temperature rise, besides releasing dissolved gases, effects vaporization of alcohol, organic acids, and water, and so replaces fermentation to a degree. Terminally, the coagulation and gelatinization become complete and permanently fix the structures.

Correlation of microscopic structures and crumb qualities: Three characteristics of the crumb are directly correlated with the microscopic findings of bread. Obviously, the coarseness of the grain is a function of the size of the gas cells: the finer the grain the smaller the gas cells. The texture basically corresponds to the average thinness of the cell walls, thin walls being "soft and silky" while thick ones feel rough and harsh. The color of the crumb is closely related to the intramural gas pockets. By analogy, other gas suspensions (*e.g.* whipped cream, whipped egg whites) become whiter as the gas bubbles become smaller and more numerous, so, in bread, the larger cells are too coarse to do this, but their walls become whiter with the appearance of numerous microscopic bubbles within them (as in Fig. 32). The grain, texture, and color of the crumb are also mutually interrelated by these intramural pockets which thin the walls by subdivision, lower the average diameter of the cells, and whiten the color. Thus, crumb quality may be accurately measured.

Summary

This paper makes reference to the lack of published information concerning the microscopic structure of breadstuffs, briefly reviews some of the previous work in the field, analytically discusses the problem of making suitable sections, and lists the highlights of the adopted microscopic procedure.

Thirty-six photomicrographs are presented and described as a series beginning with the ingredients, following with the progression of dough samples taken after each operation, and terminating with the baked bread. Three routine magnifications were used in order to include as many of the changes as possible. The descriptions concern the starch, yeast, gluten, fatty substances, and the gas cells produced by leavening.

The photomicrographs are discussed under six subdivisions:

1. *Mixing* results in an entirely homogeneous suspension of starch, yeast, and fats in a glutenous matrix. Flour particles lose their autonomy.

2. *Processing* improves the bread by grouping the starch and more efficiently sealing the gas pockets by gluten segregation. Molding and proofing are fundamental operations.

3. *Baking* changes, involving oven spring, starch gelatinization, coalescence of the lipids, and leavening are visually demonstrated in the photomicrographs.

4. *Shortening* distribution in bread dough is indicated by the technique used. The observations suggest a concentration of the shortening upon the starch-gluten interfaces, effected principally by mixing, and coalescing during baking into sizable collections of specifically stained material.

5. *Leavening* involves a preliminary diffusion away from the point of CO_2 formation. The heat of baking affects gas solubility, vaporization, enzymatic behavior, and gluten properties.

6. *The crumb qualities* (grain, texture, and to some extent color) are directly correlated with microscopic structures, particularly the small intramural gas cells.

Acknowledgments

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THE TESTING OF WHEAT QUALITY BY RECORDING DOUGH MIXER CURVES OBTAINED FROM SIFTED WHEAT MEALS [1]

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Cereal investigators have attempted to devise numerous "short-cut" methods for testing wheat quality. A method which does not require refined flour is of distinct advantage as it eliminates the process of milling. Furthermore, a method that would not require the amount of material usually needed in experimental milling would be desirable for evaluating new wheat varieties early in a plant-breeding program. A rapid, reliable test for inherent quality of wheat would also be valuable to grain buyers.

[1] Contribution No. 77, Department of Milling Industry.

Review of Short Methods

The wheat-meal-time-fermentation test has been studied by Saunders and Humphries (1928), Cutler and Worzella (1931), Pelshenke (1933), Bayfield (1935), Swanson (1937), Swanson and Dines (1939), and others. The test, although a rapid method, is limited in scope because of the many factors affecting the time or the interval between placing the dough ball in the water and the observance of disintegration. Methods of tempering and grinding the meal, because of their effect on granulation, were found to influence the time. Proteases, protease activators, and wheat germ shortened the time, while the addition of bran made it longer. Bayfield (1935) pointed out that heat treatment of samples influences the time test.

The application of viscometric technique to wheat meals has been investigated by Morgan (1924), Durham (1925), and Bayfield (1932). Morgan reduced the wheat to floury fineness by two or three reductions on an Arcade flour mill. The method of reduction employed by Durham consisted of several grindings with an Enterprise coffee mill and siftings over 34 wire and 10 XX flour cloth. The final overs of the 34 wire and 10 XX flour cloth were passed between smooth rolls and again sifted over the same sieves. The resulting meal was relatively free from bran. Bayfield employed a hammer type of mill for reducing the wheat to floury granulation.

Geddes and Aitken (1935) described a micro milling and baking technique which has proved valuable in the testing of small samples of grain. Only 100 g of wheat for milling and 25 g of flour for a single bake were required. This method of testing small samples has been adopted at the North Dakota Agricultural Experiment Station (Harris and Sanderson, 1939).

The use of recording dough mixers in the study of mixing behavior of flours is now familiar to cereal workers. Although the application of these recording mixers has been limited almost entirely to refined flours, they also have been used on wheat meals. Malloch (1938), using the Malloch recording dough mixer, found a significant correlation ($r = +.88$) between the breaking point of meal doughs and flour doughs. This relationship was deemed valuable in an approximate classification of wheat samples. Rowland Clark [2] (unpublished data) has been using the National Swanson-Working micro recording dough mixer on meals obtained by grinding wheat on a Labconco mill and sifting through a 10 XX flour cloth. The advantages of a short process of preparation in testing small samples are obvious. Since it was found

[2] The authors wish to acknowledge valuable suggestions received from Mr. Clark, Chief Chemist, Shellabarger Mills, Salina, Kansas.

that preliminary treatments of the wheat samples, such as methods of grinding and sifting as well as water absorption, influence curve characteristics, it was decided to study these factors.

Methods

Two wheat varieties, Tenmarq and Chiefkan, both of high protein content and producing curves of widely different characteristics, were chosen for the studies of the effect of grinding, tempering, and absorption upon the type of curves produced. Flours from these two varieties were milled on the 65-barrel Kansas State College mill while a Hobart mill was employed to grind small portions of these wheat meals for use in the recording dough mixer.

The method of regulating the fineness of grinding with the Hobart mill was as follows: The burr gauge was placed on the 1½ mark and the center setscrew tightened until the burrs touched slightly when the grinder was in motion. This was considered as the initial standard setting. Moving the burr gauge to the No. 1 position gave the finest setting used, while moving to No. 2 gave the coarsest. The ground meal was sifted with a small rotary sifter, using a stack of sieves as in the bolting of flour. The amount of sifting for each operation and sieve system was measured by means of a time switch attached to the rotary sifter. Percentage of extraction was calculated on the basis of the clean air-dry wheat.

The curves were made by the National Swanson-Working micro recording dough mixer, using No. 9 spring setting. Both the flours and meals were weighed out to give 35 g of material on the 15% moisture basis. The amount of water required for optimum consistency for flours as well as meals was read from a graph in which protein content was plotted against absorption. This graph was constructed as follows: The proper absorption for both the low (near 9.5%) protein and the high protein content samples of each variety was carefully determined by the manual method. The two absorption values were plotted as ordinates and their respective protein contents as abscissas and a straight line drawn between the points thus established. The position of the protein figure on this line was then used as an indication of the proper absorption for the corresponding sample. Samples below 9.5% protein were treated as if they contained 9.5% protein. This was done since the linear relation between protein content and absorption does not seem to continue below 9.5% protein. Markley, Bailey, and Harrington (1937) have demonstrated the linear relationship between protein content and absorption. Karl F. Finney [3] (unpublished data) uses such a method for estimation of absorption in the baking test.

[3] Baking Technologist, Hard Winter Wheat Quality Laboratory, Manhattan, Kansas.

Experimental

In Figure 1 are shown the effects of absorption, grinding, and tempering upon the characteristics of the dough mixer curves. Curves 1, 2, 3, 4, 5, and 6, were prepared from Tenmarq and Chiefkan flours, as

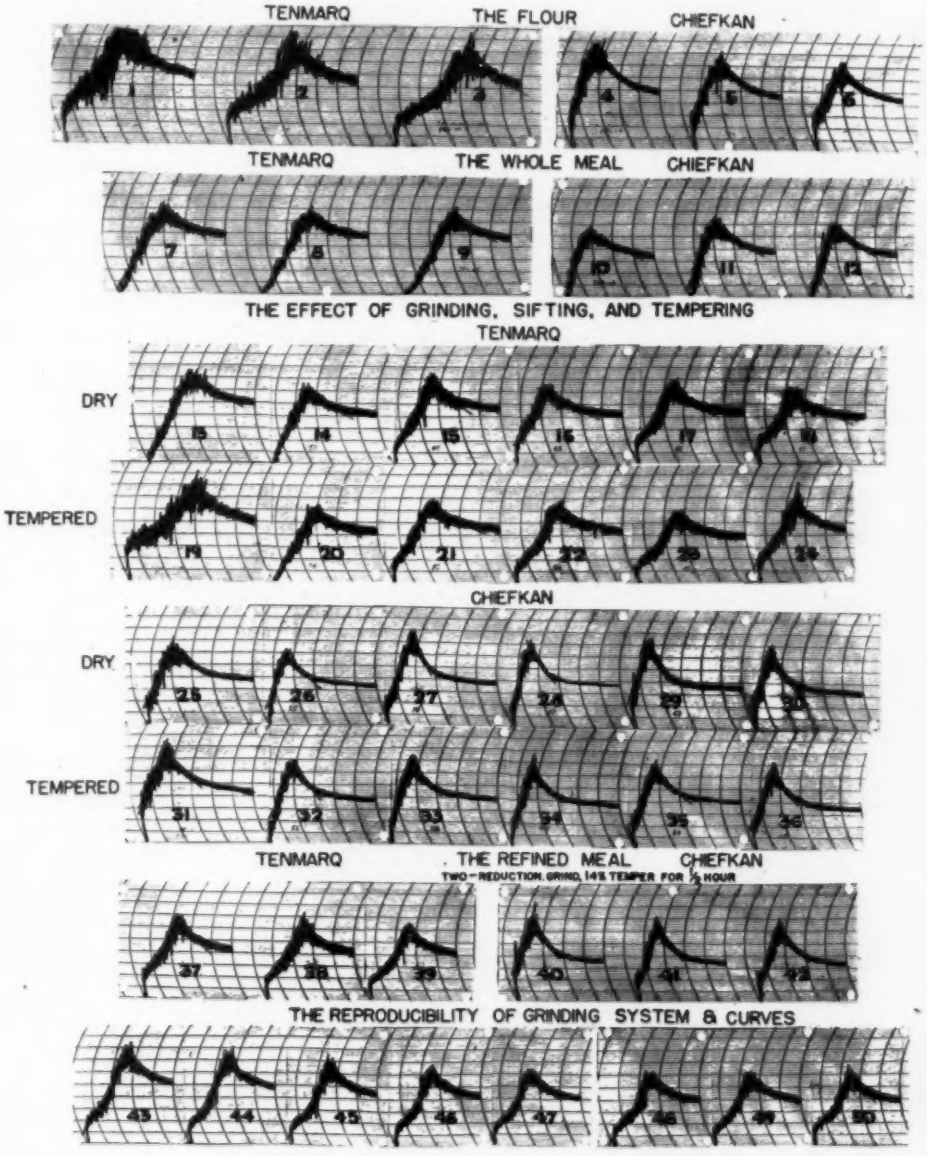

Fig. 1. Effects of grinding, sifting, and tempering on mixing-curve characteristics.

indicated in the figure, arranged in order of increasing absorption at 2% intervals starting at 63%. Directly below these curves are shown the corresponding whole-wheat-meal curves, ground only once through the Hobart mill with the finest setting. It is apparent that the whole-wheat-meal curves did not give quite as large differentiation between varieties as the flour curves. The meals were less sensitive to the amount of absorption and the differences in the time of development between the Tenmarq and Chiefkan were less than for the corresponding flours.

The effects of tempering, grinding, and sifting the wheats are shown in curves 13 to 36. Curves 13 to 18 are from Tenmarq wheat ground air-dry on the Hobart mill, sifted, and arranged in order of increasing fineness. Curve 19 was made for comparison from the Tenmarq flour using an optimum absorption. Curves 20 to 24 correspond to curves 14 to 18 with the exception that a 14% temper for one-half hour was used before grinding. Curves 18 and 24, obtained by a two-reduction grinding which resulted in the finest meal, approached No. 19 more closely. Tempering had comparatively little effect upon the character of a curve except a slight tendency to increase the time of development. The Chiefkan curves, 25 to 36, were obtained similarly to those of Tenmarq. No. 31 being made from the flour. These show, in comparison with the Tenmarq curves, 13 to 24, nearly as great a differentiation between these two varieties as is shown by the flour curves 19 and 31, respectively.

Curves 37 to 42 are from sifted meals of Tenmarq and Chiefkan, respectively, prepared by a two-reduction grinding with a 14% temper for 30 minutes and arranged in order of increasing absorption of 2% intervals starting at 70%. While increased absorption caused a slightly longer time of development and decrease in the height of curve, the differentiations between varieties shown by curves 37 to 39 (Tenmarq) and 40 to 42 (Chiefkan) are practically as great as shown between 1 to 3 and 4 to 6, made from the corresponding flours.

The reproducibility of curves from a two-reduction grinding and bolting system is shown in curves 43 to 47, which are arranged in order of grinding. Curve 43 was made from meal obtained on a cold mill. With continued grinding the mill becomes warmer and the effect is shown in the character of the curves being regularly reduced in curve height. This indicates that the curves from a meal ground on a cold mill will not be the same as when ground on a warm mill. For this reason a large sample of grain should be ground before grinding the test samples. Curves 48 to 50 were made from meal ground as one large sample and show the uniformity in curves when there is no variation in grinding.

Effects of Tempering

The effects of tempering are shown in Figure 2 and the corresponding data are given in Table I. The wheat samples were tempered to 11%, 12%, 13%, and 14% for 15, 30, and 60 minutes. In Table I the column headings under "Data from curves" are explained as follows:

TABLE I

THE EFFECT OF TEMPERING WHEAT UPON THE RESULTANT DOUGH MIXER CURVES

Variety	Curve No. (Fig. 2)	Treatment		Grind extraction	Analytical data			Data from curves		
		Time	Temper		Moisture	Ash	Absorption	Time of development	Curve height	Angle of slope
		min	%	%	%	%	%	*min*	*units*	*deg*
Tenmarq	1	0	0	38.0	9.5	0.87	67.1	2.4	7.1	74
"	2	15	11	48.0	10.3	0.96	68.7	2.5	6.8	75
"	3	15	12	47.3	10.7	1.01	69.4	2.6	7.0	71
"	4	15	13	41.3	12.0	1.02	71.9	2.6	7.1	80
Chiefkan	5	0	0	48.0	9.4	0.90	64.3	1.5	6.5	63
"	6	15	11	48.0	10.5	0.95	66.2	1.6	6.6	58
"	7	15	12	50.1	11.0	0.97	67.1	1.6	6.5	63
"	8	15	13	50.0	11.6	0.98	68.2	1.6	6.1	66
Tenmarq	9	30	11	52.7	11.0	0.97	69.8	2.6	6.8	81
"	10	30	12	51.3	11.2	0.99	70.4	2.5	6.5	81
"	11	30	13	50.0	11.6	0.99	71.1	2.7	6.1	87
"	12	30	14	50.7	13.0	1.02	73.9	2.9	6.6	88
Chiefkan	13	30	11	50.0	10.3	0.95	65.8	1.4	6.3	61
"	14	30	12	50.0	10.9	0.97	66.9	1.4	6.6	56
"	15	30	13	47.3	11.8	1.01	68.6	1.6	6.4	65
"	16	30	14	51.3	12.1	1.03	69.2	1.5	6.8	61
Tenmarq	17	60	11	44.7	10.6	0.93	69.7	2.6	6.2	78
"	18	60	12	49.3	11.0	0.92	70.5	2.6	6.2	84
"	19	60	13	50.7	12.0	0.94	72.4	2.7	6.7	74
"	20	60	14	57.3	13.1	1.03	74.6	3.1	6.8	79
Chiefkan	21	60	11	47.3	10.1	0.95	65.5	1.5	6.9	50
"	22	60	12	46.3	10.4	0.97	66.0	1.4	6.5	55
"	23	60	13	52.0	11.1	0.99	67.3	1.6	6.7	56
"	24	60	14	49.3	13.4	1.04	71.8	1.9	6.7	57

Time of development is determined by the number of curved vertical lines upon the chart paper passed before the dough reaches its maximum resistance to the revolving pins. The time to pass between two curved lines is about one minute. Height is given in the number of horizontal lines on the chart paper that are passed when the top is reached. Angle of slope is the number of degrees obtained by measuring the angle between lines drawn in the center of the upslope and the first part of the downslope.

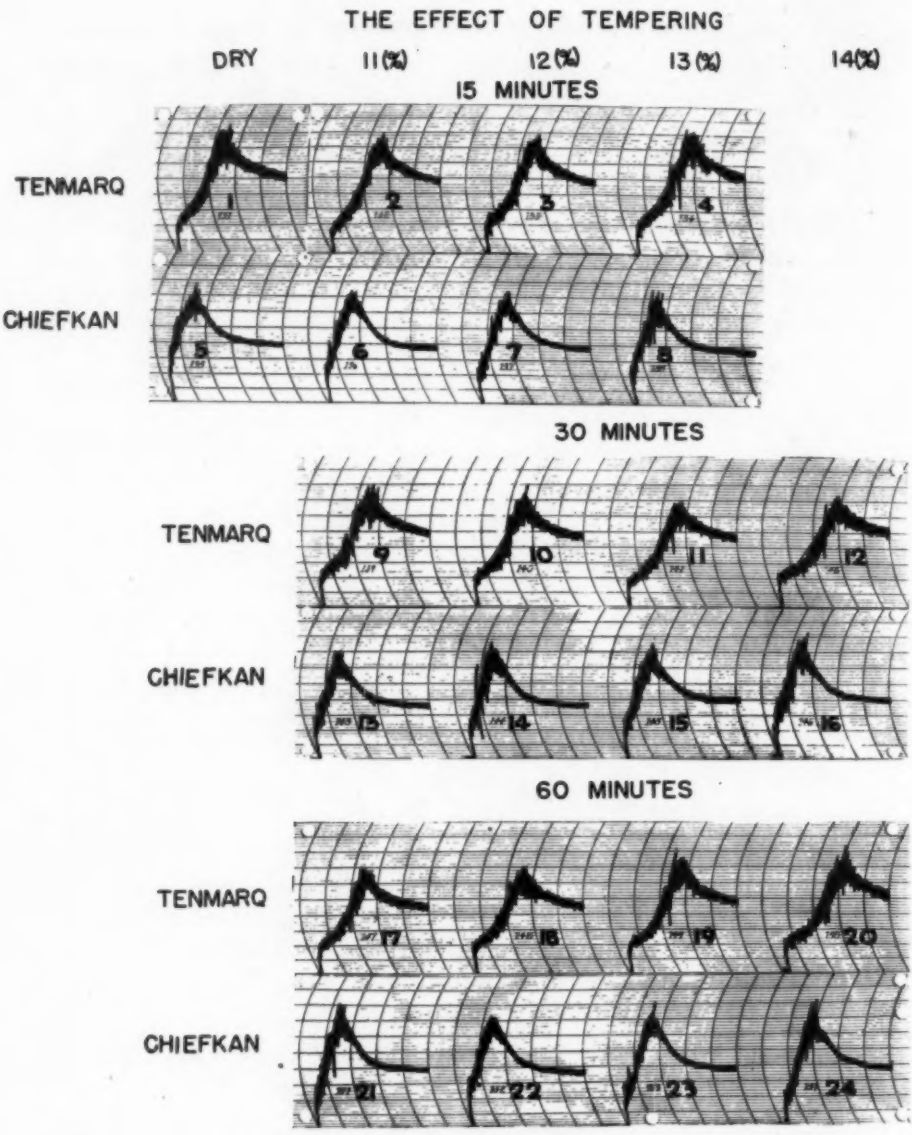

Fig. 2. Effect of tempering on mixing curve characteristics.

The curves in Figure 2 indicate that the percent and length of temper had but little effect on the curve characteristics. Data in Table I also indicate that the length of temper had very little effect, but the amount of tempering water increased the moisture and ash contents and tended to increase the percentage of extraction as well as the absorption slightly.

With increased amount of tempering water and consequent larger moisture content, the time of development tended to be lengthened, but the heights of the curves and angles of the slopes indicate no definite trend. It appears that any tempering treatment, within the range studied, would aid in grinding without materially affecting the curve characteristics. It is very likely that a more refined meal could be obtained if the wheat were allowed to be tempered for a longer period, such as is customary in roller milling, but this would be impractical in this type of testing.

The system finally adopted in preparation of the sifted meals is as follows: The whole cleaned wheat, tempered to 14% moisture for 30

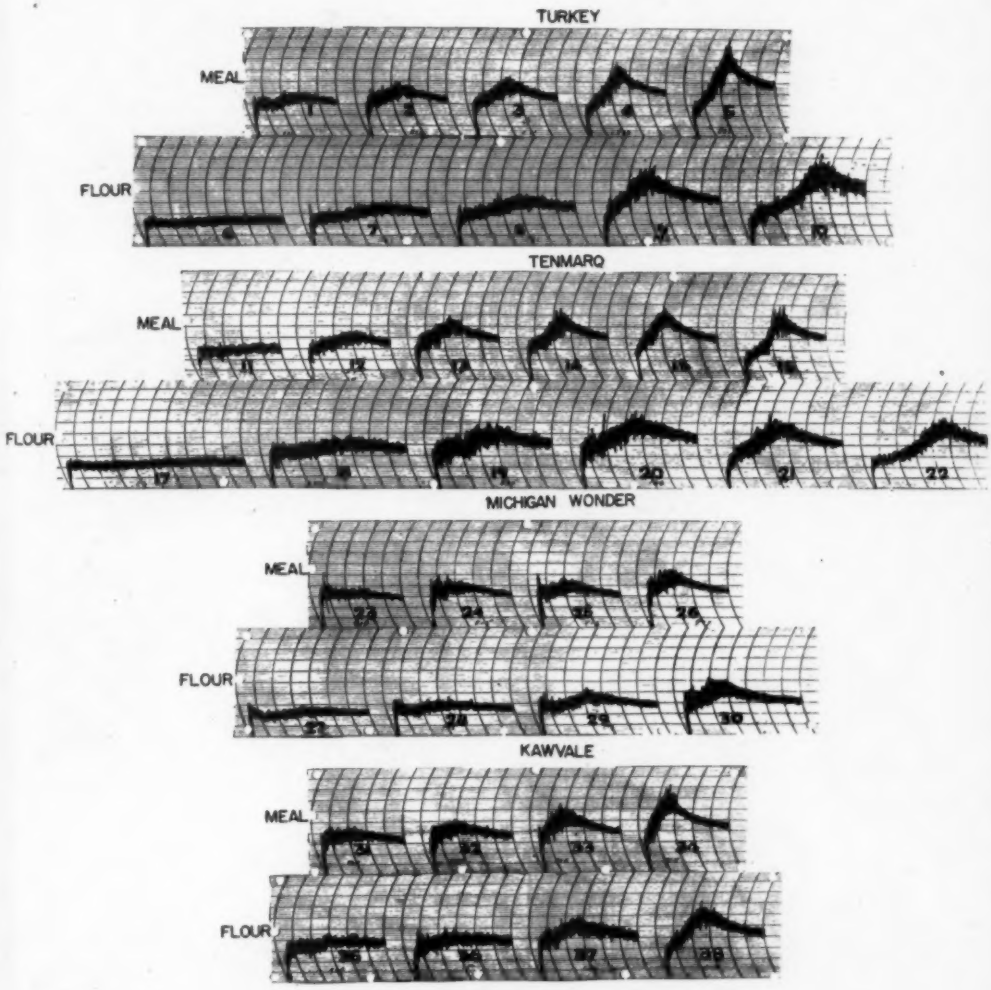

Fig. 3. Comparisons of meal and flour curves.

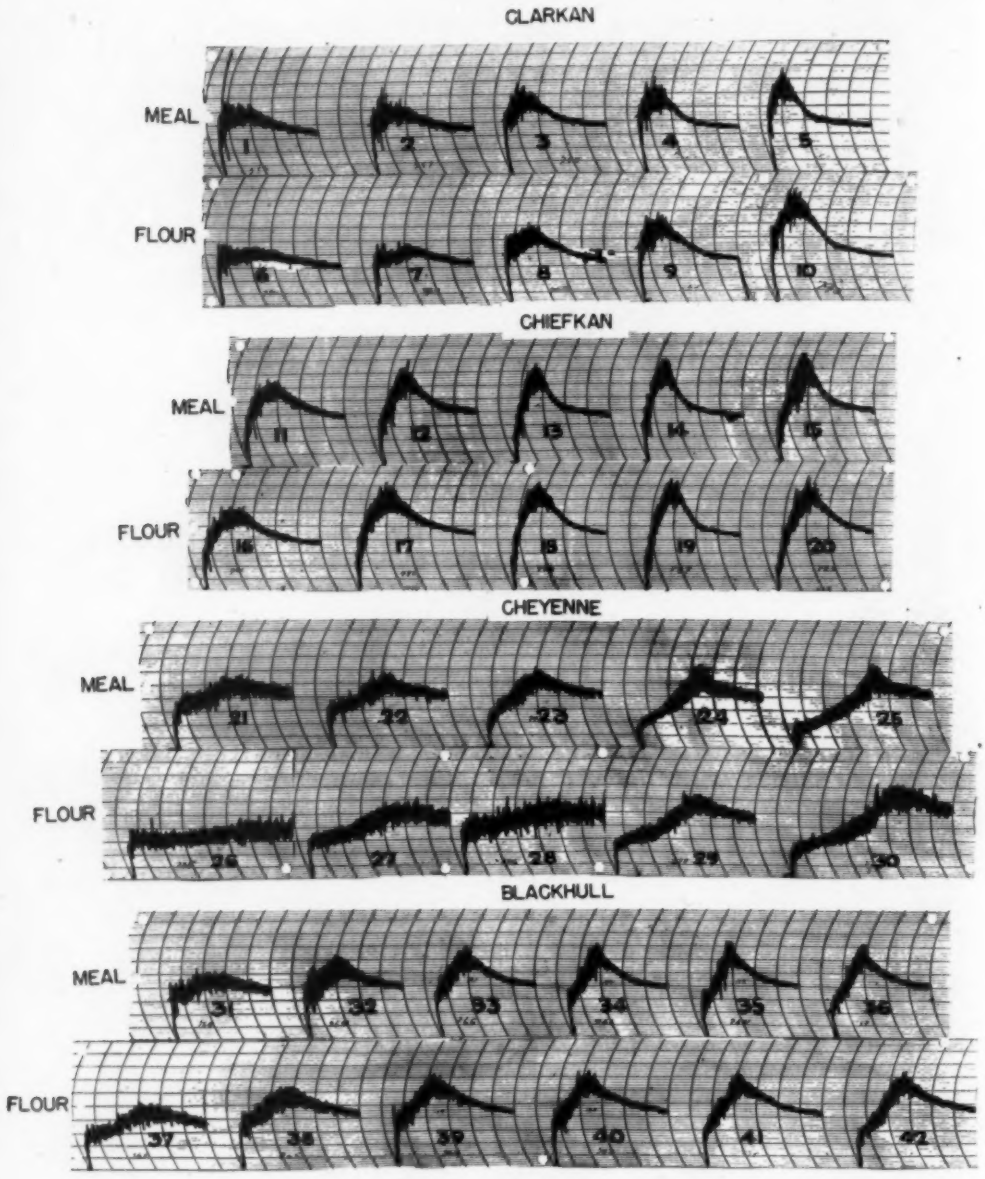

Fig. 4. Comparisons of flour and meal curves.

minutes, is ground, using the No. 2 setting on the Hobart mill. The ground meal is then sifted for four minutes, using the following stack of sieves: 26 wire, 50 GG, 70 GG, and 10 XX. The overs of the 26 wire are discarded. The overs of 50 GG and 70 GG are reground separately,

using the No. 1 setting, and again sifted over the 50 GG, 70 GG, and 10 XX for four minutes, the overs of the 50 GG and 70 GG being discarded. The overs of the 10 XX are reground, using the No. 1 setting and resifted on 10 XX flour cloth, the overs being discarded. The throughs of the 10 XX make up the meal used for making the curves. These meals are not free from bran specks, as might be expected, but the fineness is quite uniform.

Comparison of Sifted Meals with the Corresponding Flours of Several Varieties

Further studies were continued in comparing the curves made from meals and the corresponding flours. Several varieties of wheat were available, each variety consisting of a series of samples varying in protein content. The flours had been milled on a laboratory Allis-Chalmers mill. The meals were prepared by the method given in the preceding paragraph. The curves obtained on these meals and flours are shown in Figures 3 and 4 and analytical as well as other data are given in Table II. The meal and flour curves for each variety are arranged in order of increasing protein content. The absorption used for each meal and flour respectively was interpolated from a protein-vs.-absorption graph as explained previously.

The data in Table II show that the meals had higher ash, higher protein, and higher absorptions but lower moisture percentages than the flours. It seems likely that the higher absorption of the meals was due partly to their higher protein content as compared with that of the flours.

The curves in Figures 3 and 4 indicate that the meal curves bring out characteristics of the wheat varieties in a way similar to the flour curves. Although the meals are slightly higher in protein content, and, as will be shown later, protein content influences certain characteristics in the curves, yet the curve characteristics of the various varieties are different for both the flour and the meal.

Correlation Coefficients

In order to test how closely the characteristics of the curves obtained from the sifted wheat meals agree with those from the flours and also to test the relation of protein content to these characteristics, the correlation coefficients were calculated and tabulated in Table III. The figures for the calculation of these coefficients were obtained from the data on the curves, meals, and flours given in Table II. Data on curves from the meals and flours of the lowest protein content were not included in the statistical study because of the uncertainty in locating suitable points of measurements.

The correlation coefficients as shown in Table III indicate the following: Protein content of the meal or flour is positively correlated with the height of curves at optimum consistency, while it is negatively correlated with the angle of slope. The protein content of the meal is posi-

TABLE II

A Comparison of Sifted Meal with the Corresponding Flour of Several Varieties

Variety	Curve No. (Fig. 3)	Material	Grind extraction	Analytical data				Data from curves		
				Moisture	Ash	Protein	Absorption	Time of development	Curve height	Angle of slope
			%	%	%	%	%	*min*	*units*	*deg*
Turkey	1	Meal	46.8	12.8	0.99	9.6	57.9	—	—	—
"	2	"	48.8	12.5	1.00	10.5	59.8	2.5	4.3	138
"	3	"	48.0	12.7	1.06	11.9	63.1	2.5	4.7	133
"	4	"	49.2	12.1	1.07	15.5	69.7	2.4	5.7	99
"	5	"	48.0	11.9	1.15	17.4	72.7	2.3	7.0	72
"	6	Flour	—	14.3	0.38	8.2	57.2	—	—	—
"	7	"	—	14.2	0.39	9.3	57.0	4.6	3.5	162
"	8	"	—	14.0	0.40	10.2	58.1	3.9	4.2	156
"	9	"	—	13.7	0.44	11.8	59.6	3.0	6.0	115
"	10	"	—	13.9	0.45	15.5	66.8	4.4	6.7	118
Tenmarq	11	Meal	53.6	12.6	1.03	9.0	57.1	—	—	—
"	12	"	46.8	12.5	1.03	10.3	58.7	3.2	4.1	149
"	13	"	47.2	12.3	1.08	12.3	62.9	2.4	5.1	125
"	14	"	46.0	12.2	1.07	13.6	65.1	2.5	5.4	115
"	15	"	48.0	12.2	1.10	14.6	66.9	2.1	5.8	106
"	16	"	46.4	12.4	1.16	17.6	73.6	2.5	5.6	96
"	17	Flour	—	14.7	0.40	7.9	57.0	—	—	—
"	18	"	—	14.3	0.39	9.3	56.2	5.2	4.0	165
"	19	"	—	14.3	0.37	10.8	58.2	4.3	5.1	145
"	20	"	—	14.2	0.38	11.8	60.0	3.5	5.8	137
"	21	"	—	14.1	0.37	13.3	62.8	3.4	5.5	128
"	22	"	—	14.0	0.43	15.3	66.0	4.8	5.6	132
Michigan Wonder	23	Meal	46.8	11.8	0.89	8.9	52.3	—	—	—
"	24	"	46.4	12.2	0.76	9.6	53.0	2.4	3.8	162
"	25	"	49.5	12.2	0.77	10.2	55.4	2.4	3.9	157
"	26	"	56.0	11.9	0.75	13.0	59.7	2.0	4.8	132
"	27	Flour	—	14.3	0.37	8.3	53.2	—	—	—
"	28	"	—	14.2	0.37	8.7	53.1	3.6	2.8	168
"	29	"	—	14.4	0.37	9.0	53.3	3.1	3.8	150
"	30	"	—	14.4	0.34	10.8	55.9	2.8	4.3	138
Kawvale	31	Meal	54.0	12.8	0.99	9.6	55.5	2.4	3.9	158
"	32	"	50.0	12.6	1.10	10.2	56.6	1.9	4.5	143
"	33	"	50.0	12.5	1.00	11.9	59.8	1.9	5.4	113
"	34	"	48.8	12.5	0.96	14.9	65.9	1.6	6.7	81
"	35	Flour	—	13.6	0.40	8.3	53.5	3.2	3.9	165
"	36	"	—	13.7	0.40	8.9	53.6	3.0	4.0	160
"	37	"	—	13.9	0.38	10.4	55.5	2.7	5.2	142
"	38	"	—	14.1	0.38	13.3	61.3	2.5	6.1	110

TABLE II.—*Continued*

Variety	Curve No. (Fig. 4)	Material	Grind extraction	Analytical data				Data from curves		
				Moisture	Ash	Protein	Absorption	Time of development	Curve height	Angle of slope
			%	%	%	%	%	*min*	*units*	*deg*
Clarkan	1	Meal	51.2	12.4	0.89	9.4	53.3	2.0	4.8	153
"	2	"	53.2	12.4	0.74	9.5	53.3	1.7	4.7	137
"	3	"	52.8	12.4	0.67	11.0	55.7	1.3	5.6	108
"	4	"	51.2	12.4	0.65	12.2	58.2	1.3	5.6	103
"	5	"	57.2	12.2	0.81	14.1	60.6	1.0	6.3	77
"	6	Flour	—	14.4	0.32	8.3	52.8	2.5	4.0	167
"	7	"	—	14.3	0.31	8.7	52.7	2.3	4.2	148
"	8	"	—	14.7	0.30	9.9	54.0	1.8	5.3	121
"	9	"	—	14.3	0.32	11.3	55.8	1.6	5.8	107
"	10	"	—	14.4	0.35	12.8	59.4	1.4	7.3	82
Chiefkan	11	Meal	44.8	12.9	1.14	11.8	60.0	1.8	5.8	102
"	12	"	44.4	12.9	1.04	13.0	62.5	1.7	6.5	79
"	13	"	45.2	12.6	1.12	14.1	64.4	1.6	6.5	59
"	14	"	46.8	12.2	1.12	15.3	66.5	1.4	6.8	55
"	15	"	45.2	12.2	1.17	15.8	67.6	1.7	7.1	60
"	16	Flour	—	14.6	0.41	9.3	55.8	2.3	5.9	105
"	17	"	—	14.3	0.37	10.7	57.8	1.9	7.1	96
"	18	"	—	14.6	0.37	11.6	59.7	1.7	7.3	76
"	19	"	—	14.4	0.39	13.1	62.3	1.8	7.5	61
"	20	"	—	14.2	0.43	14.6	64.5	1.9	7.4	78
Cheyenne	21	Meal	45.0	12.2	0.98	10.6	57.3	—	—	—
"	22	"	43.2	12.6	1.04	12.5	62.4	3.3	4.9	138
"	23	"	48.0	12.2	1.04	12.9	62.6	3.1	5.3	135
"	24	"	46.4	12.6	1.17	15.3	69.2	3.5	5.4	113
"	25	"	49.2	12.5	1.15	16.7	72.0	4.4	5.3	125
"	26	Flour	—	14.3	0.37	9.2	55.8	—	—	—
"	27	"	—	14.3	0.37	10.4	57.2	4.8	5.2	148
"	28	"	—	14.2	0.40	11.2	59.0	5.0	5.2	159
"	29	"	—	13.7	0.44	13.2	61.0	4.5	5.7	134
"	30	"	—	14.5	0.44	14.8	65.5	5.5	6.6	130
Blackhull	31	Meal	54.4	12.6	1.09	9.4	55.1	2.3	4.7	141
"	32	"	53.2	12.4	0.98	10.3	56.2	2.1	5.4	109
"	33	"	52.2	11.8	0.86	11.9	59.0	1.9	6.0	99
"	34	"	52.8	12.4	0.91	13.2	63.5	1.8	6.2	88
"	35	"	52.0	12.2	0.90	14.5	66.0	1.7	6.3	79
"	36	"	52.0	11.5	0.92	16.6	69.5	1.7	6.3	80
"	37	Flour	—	14.6	0.35	8.4	55.3	3.3	4.5	143
"	38	"	—	14.4	0.35	9.1	54.9	2.8	5.7	128
"	39	"	—	14.2	0.35	10.5	56.5	2.3	6.4	106
"	40	"	—	14.2	0.36	11.8	59.0	2.3	6.5	99
"	41	"	—	14.3	0.38	13.1	61.7	2.2	6.4	95
"	42	"	—	14.1	0.41	15.1	65.2	2.5	6.5	101

tively correlated with that of the flour. In this study no significant correlation was demonstrated between protein content and the time of developmenet as measured to the peak of the curve. At low protein

TABLE III

CORRELATION COEFFICIENTS COMPUTED FROM ANALYTICAL AND CURVE DATA

$N = 35$

Variables correlated		Correlation coefficient
X	*Y*	*rxy*
Meal, protein (%)	Flour, protein (%)	**+.9623**[1]
Meal, protein (%)	Meal, time of development (*min*)	+.1324
Flour, protein (%)	Flour, time of development (*min*)	+.1070
Meal, protein (%)	Meal, height of curve (*units*)	**+.7207**
Flour, protein (%)	Flour, height of curve (*units*)	**+.6863**
Meal, protein (%)	Meal, angle of slope (*deg*)	**−.7097**
Flour, protein (%)	Flour, angle of slope (*deg*)	**−.5841**
Meal, angle of slope[2] (*deg*)	Flour, angle of slope[2] (*deg*)	**+.9221**
Meal, time of development (*min*)	Flour, time of development (*min*)	**+.8738**
Meal, height (*units*)	Flour, height (*units*)	**+.9159**

[1] Bold type correlations are highly significant, since with 1% level $rxy = 0.425$.
[2] Angle of up-and-down slopes.

levels the time of development might appear to be longer than at higher protein levels. However, as it was impossible with the lowest protein samples to determine this peak from the curve accurately, these samples were not used in calculating the correlation coefficients. While the time of development as shown in Table II indicates a trend to decrease with increasing protein in all varieties except Cheyenne, this trend is not uniform and therefore the correlation coefficient between these variables is not significant. Of most interest is the existence of a high positive correlation between curve measurements of the meal and of the flours in respect to time of development, height of curve, and angle of the up and down slope. While these meal and flour curve characteristics are highly correlated, they are not identical, but the differences between them vary together. The meal curves in comparison with the flour curves tend to have a shorter time of development, a smaller up and down slope angle, and about the same height. It is apparent from Figures 3 and 4 that not all varieties tended to give curves in which the co-variance of meal and flour characteristics are the same. The meal curves of Clarkan, Chiefkan, and Blackhull show smaller differences in the time of development in comparison with the flour curves than do the other varieties. Tenmarq and Cheyenne show the greatest difference in this respect. The varietal factors must be considered when interpreting the meal curves.

Since protein content of the meal is highly correlated with protein content of the flour and since the protein content is positively correlated with the height of the curves and negatively correlated with the angle of slopes and also since the meal curves are correlated with the flour curves, it appears that the meal curves may be used as an approximate indication of the protein content, provided the time of development is taken into account. A classification of wheats into high, medium, and low protein

content can be made on the basis of curve characteristics. Furthermore, since the time of development varies more than any of the other characteristics among the varieties and also since this time is not correlated with the protein content, the time of curve development can be used to characterize the varieties in a general way. With the possible exception of Cheyenne the varieties of longer mixing times are generally considered to have a wider range of adaptability for bread-making.

Conclusions

A method has been described which makes it possible to obtain satisfactory curves on the recording dough mixer, using sifted wheat meals instead of flours. While the curves obtained from the meals are not exactly like those obtained from the flours, yet they are closely correlated in the characteristics of height, time of development, and the angles made by the up and down slopes of the curves. These correlations are statistically significant and hence the dough-mixer curve characteristics of a wheat may be observed almost as well from the meal curve as from the flour curve.

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THE FERMENTOMETER, A GAS-PRODUCTION MEASURING DEVICE

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Various types of pressure recording instruments for measuring gas production in doughs have been built. Sandstedt and Blish (1934) proposed the use of a "pressuremeter." Bailey (1939) suggested an apparatus which indicated both gas production and gas losses in fermenting doughs. Eisenberg (1940b) suggested another type of volumetric gas measuring apparatus. Other methods for volumetric measurement of this factor have been described by Brabender (1933) and Elion (1933). Eisenberg (1940a) doubts the contention of Sandstedt and Blish (1936) that gas production under pressure proceeds at the same rate as at atmospheric pressure.

Materials and Methods

A manometric apparatus was constructed embodying the principles used by Sandstedt and Blish in their pressuremeter and by Bailey in his gas-production and gas-retention apparatus (Fig. 1). It consists of a mercury manometer and a meter stick mounted on a brass plate, which has a rubber gasket the size of the top of a pint Mason jar countersunk into its underside. The fermentation chamber is the pint-size special-glass jar. This rests in a stand made of a brass plate with threaded rods mounted vertically in each corner. The apparatus has the advantage of using the idea of Bailey for the determination of the gas retention factor as well as the total gas production factor. This can be accomplished by using measured quantities of solutions of sodium chloride for total gas produced, or sodium hydroxide for gas retained. The dough is put into a screen-wire receptacle which in turn rests on a heavy copper wire base which keeps the wire basket above the solution.

In operation, the jar containing the dough and the proper solution are placed into the stand with the manometer set down over it and carefully adjusted so that the top of the jar and the rubber gasket meet exactly. These are then brought together tightly by means of wing nuts turning on the threaded rods set in each corner of the brass plates.

Doughs are made from 200 g of flour containing 15% moisture (or its equivalent in dry weight), 120 ml water, and 6 g of yeast. Other ingredients are added by dry mixing with the flour. Ten percent aliquot fractions of dough are weighed off carefully and placed into the dough baskets. All fermentation tests are carried out at a constant

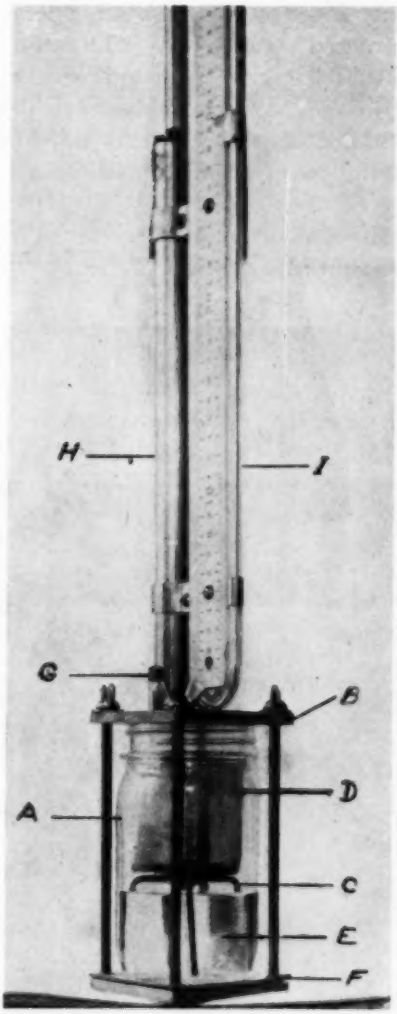

Fig. 1. The fermentometer: *A*, pint-size glass jar; *B*, brass plate 3″ x 3″ with rubber gasket countersunk in underside; *C*, heavy copper wire stand; *D*, screen wire (40-mesh) dough basket; *E*, solution—23% NaCl or 23% NaOH; *F*, rubber gasket; *G*, automobile tire valve for releasing pressure; *H*, brass pipe ¾″; *I*, mercury-filled glass manometer.

temperature of 30°C by means of a water bath. Mixing, dough scaling, and mounting operations take about 6 minutes per dough. About 9 minutes are allowed for the apparatus and the dough piece to come to temperature. Exactly 15 minutes after the flour and the yeast water are first mixed, the mercury in the manometer is adjusted to 0 by venting the gas in the fermentation chamber by means of a valve in the top plate of the apparatus.

In this laboratory a set of eight units is operated simultaneously in a specially constructed water bath. The latter is a stainless steel box 12″ × 14″ × 10″ deep. It is fitted with a thermo-regulator, knife-blade heater, cooling coils, and mechanical stirring apparatus. In the water bath the units rest on racks which raise them an inch above the floor of the bath, permitting thorough circulation of the water (Fig. 2).

Readings in millimeters of pressure are taken hourly. The results are calculated by means of Boyle's law to the amount of carbon dioxide

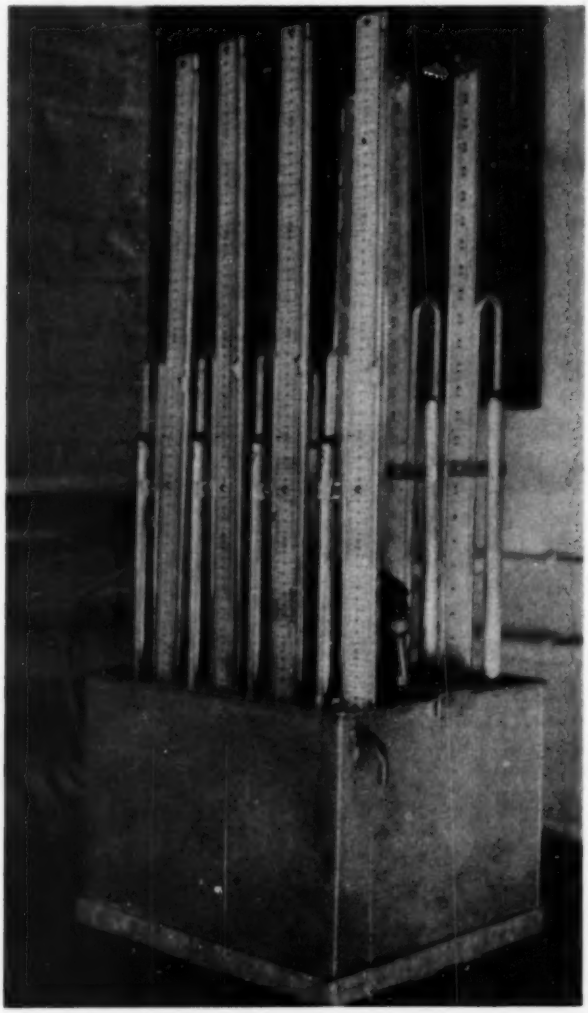

Fig. 2. Eight fermentometer units in a stainless steel constant-temperature bath.

gas evolved by 100 g of flour at 15% moisture at 760 mm pressure and 30°C.

Calibration of the Apparatus

A series of seven doughs were made in the manner described in the foregoing paragraphs, using a well blended flour and a standard yeast. Duplicate aliquots were taken from each dough and gas production determined. Readings were taken at each half hour for five hours. The composite results of these 14 determinations are given in Table I.

TABLE I

GAS PRODUCTION VALUES

Time	Average, 14 determinations	Variation, 14 determinations
hrs	*ml*	*ml*
½	179	±14.0
1	341	±17.0
1½	615	±31.0
2	907	±40.0
2½	1170	±25.0
3	1314	±13.5
3½	1399	± 7.5
4	1466	± 8.5
4½	1520	± 8.0
5	1568	±17.0

From Table I it will be noted that variation is greatest at the 1½, 2, and 2½ hour readings. The data also bring out the fact that variation is least at the period of final readings (4 and 4½ hours). The 4½-hour period has been chosen by this laboratory as the point for total gas production readings since at this point the degree of accuracy of this apparatus is greatest.

Effect of Varying Pressure on Gas Production

The following experiments were made on four doughs with duplicate portions from each, making a total of eight determinations for each experiment. Readings were taken hourly to 4 hours and again at 4½ hours, under conditions, respectively, as follows: (1) gas pressure allowed to build up for the full 4½ hours; (2) gas pressure released at the end of each one-hour period, apparatus reset to zero; (3) tests started at pressure of 100 mm by pumping air into the units after sealing, pressure allowed to build up for the full period of 4½ hours; and (4) the same as (3), except that the pressure was reset each hour to the starting point of 100 mm. The results in ml per 100 g flour at 15% moisture are shown in Figure 3. It will be noted that the curve representing Experiment 1 shows the highest gas volumes throughout the test period. There was, however, no significant difference between

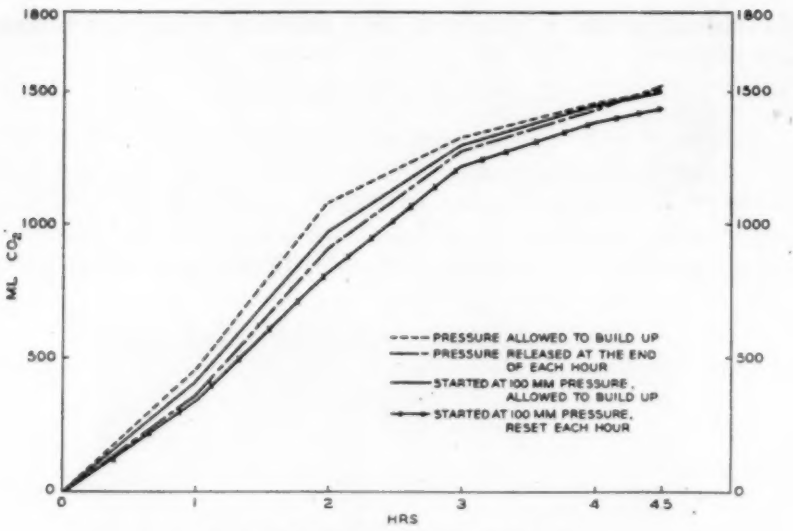

Fig. 3. Effect of varying pressure on gas production.

the final readings of this experiment and those of Experiments 2 and 3. The greatest variation between experiments (260 ml) was at the second hour reading. Experiment 4 showed the only significant differences throughout the period of the test.

Summary

A new variation of the pressuremeter, the fermentometer, is described. This apparatus is designed to register gas production and gas retention during dough fermentation. Calibration and degree of accuracy data on gas production are given.

Curves indicating the effect of pressure on gas production in dough are presented.

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BROMIDE RESIDUES IN CEREALS FUMIGATED WITH METHYL BROMIDE

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Since the excellent pioneer work of Mackie (1937, 1938), Hawkins (1938, 1939) and their collaborators, methyl bromide has been widely accepted for the fumigation of nursery stock and fresh or dried fruits and vegetables. In view of the economy, efficiency, and thoroughness of penetration of this fumigant, much interest has been aroused concerning its use in the cereal industry. However, the finding of somewhat higher bromide residues in certain products such as milled grains after fumigation on a laboratory scale (Stenger, Shrader, and Beshgetoor, 1939; Dudley *et al.*, 1940) has raised a question as to whether such residues might be objectionable in the fumigated products (Cotton, 1941). Until recently it had been feared that the residual bromide might consist in part of free methyl bromide, no method having been available for distinguishing between the various forms of bromide present in these small amounts.[1] Now a method for this purpose has been developed and the bromide remaining in fumigated flour has been shown to be largely inorganic immediately after fumigation and entirely inorganic within several days (Shrader, Beshgetoor, and Stenger, 1941). Thus any fear of methyl bromide remaining in the product has been dispelled.

It is the purpose of this paper to present additional information regarding the bromide residues in flour and other cereals, and to show that not only do the residues consist of inorganic bromides, but the magnitudes of these residues are lower under the recommended commercial fumigation conditions than had previously been expected from experiments conducted on a laboratory scale. The amounts of bromide retained by a fumigated product depend greatly upon the nature of the material and upon the conditions under which fumigation takes place, in particular upon the concentration of methyl bromide, ratio of methyl bromide to product, time of exposure, temperature, type of fumigation (vacuum or atmospheric), and relative humidity in the case of the atmospheric type. To duplicate commercial conditions in the laboratory is therefore difficult.

[1] Following the completion of the experimental work here reported, there has been published a paper by E. P. Laug (1941). Although Laug concludes correctly that only nonvolatile bromide remains after a sufficient time of airing, his method for determining volatile bromide is open to question. Furthermore, his laboratory-scale tests on cereals were made almost exclusively with such a high concentration of methyl bromide (about 16 lbs per 1,000 cu ft) and high ratio of fumigant to product (apparently on the order of 500 lbs CH_3Br to 1,000 lbs of product) that the results have scarcely any significance for commercial purposes.

TABLE I

BROMIDE CONTENT OF FUMIGATED WHEAT PRODUCTS

Product and package	CH_3Br per 1,000 cubic feet	Approx. load	CH_3Br per 1,000 lbs load	Temp.	Percent bromide							
					Control		Outer layer of bag		Center of bag		Composite	
					Total	Inorg.	Total	Inorg.	Total	Inorg.	Total	Inorg.
	lbs	*lbs*	*lbs*	°F	%	%	%	%	%	%	%	%
White flour (100-lb cloth bags)	1.07	48,200	0.104	74–84	.0010	—	.0021	—	.0028	—	.0028	—
	1.28	48,200	0.125	76	.0009	.0009	.0031	.0033	.0023	.0023	.0025	.0027
	1.49	84,000	0.083	51–77	.0001	—	.0043	—	.0018	—	.0023	—
Whole-wheat flour (2.5-lb paper bags, in cartons	1.07	48,200	0.104	74–84	.0003	—	—	—	—	—	.0028	—
	1.28	48,200	0.125	76	.0002	.0003	—	—	—	—	.0038	.0038
	1.49	84,000	0.083	51–77	.0003	.0002	—	—	—	—	.0027	.0025
Whole-wheat flour, extra fine (100-lb cloth bags)	1.49	84,000	0.083	51–77	.0002	—	.0044	—	.0022	—	.0022	.0024
100% wheat germ (2-lb paper bags)	1.28	48,200	0.125	76	.0008	.0008	—	—	—	—	.0101	.0096
Wheat bran (100-lb cloth bags)	1.07	48,200	0.104	74–84	.0003	—	.0018	—	.0015	—	.0013	—
	1.49	84,000	0.083	51–77	.0002	—	.0061	—	.0040	—	.0047	—
Whole grain (soft red winter wheat, 100-lb cloth bags)	1.07	48,200	0.104	74–84	.0006	—	.0009	—	.0008	—	.0008	—
	1.49	84,000	0.083	51–77	.0002	—	.0012	—	.0006	—	.0009	.0009
Wheat shorts (100-lb cloth bags)	1.28	48,200	0.125	76	.0004	.0004	.0036	.0037	.0027	.0023	.0028	.0024
	1.49	84,000	0.083	51–77	.0003	—	.0045	—	.0075	—	.0050	—

In Table I are shown data obtained during commercial atmospheric fumigations of various wheat products. The vault employed had a capacity of 4,700 cubic feet and in each case was well loaded. The fumigation time was 24 hours and the insect kill was consistently 100%. (In a few other experiments not reported here these dosages were found insufficient to kill all insects at considerably lower temperatures.) The samples for analysis were placed in sealed containers, shipped to the laboratory, and analyzed after standing open for one week in the same containers. Analyses for total and inorganic bromides were made by the procedure of Shrader, Beshgetoor, and Stenger (1941). An experimental error of ±0.0005% bromide in any result is possible.

Since the amounts of bromide retained by white flour and whole-wheat flour have been reported as from 0.0042% to 0.0091% (Dudley *et al.*, 1940; Stenger, Shrader, and Beshgetoor, 1939; Cotton, 1941) on the basis of laboratory tests, it is evident from Table I that the retentions are generally lower following commercial fumigations. The reason for this difference may be illustrated as follows: In laboratory fumigations one usually treats small quantities of product, say a pound, in a drum with a capacity of perhaps 7 cubic feet. To obtain a dosage of one pound of methyl bromide per 1,000 cubic feet, 0.007 pound of fumigant would be needed, or a ratio of 7 pounds to 1,000 pounds of product. Absorption of part of this methyl bromide would not change its concentration appreciably. In contrast, the ratios employed in the commercial fumigations listed in Table I were on the order of 0.11 pound of methyl bromide or less to 1,000 pounds of product. Partial absorption of this lowers the concentration considerably,

TABLE II

RESIDUAL BROMIDE IN FUMIGATED CEREAL PRODUCTS

Prepared dry cereal breakfast-food products	Bromide before fumigation		Bromide after fumigation	
	Total	Inorganic	Total	Inorganic
	%	%	%	%
A Wheat	.0011	.0014	.0025	.0024
B Corn	.0011	.0015	.0020	.0028
C Bran	.0011	.0012	.0027	.0027
D Wheat	.0011	.0012	.0018	.0017
E Wheat	.0007	.0012	.0021	.0025
F Mixed grains	.0005	.0012	.0011	.0014
G Mixed grains	.0010	.0019	.0024	.0024
RAW CEREALS				
H Rolled oats	.0010	.0014	.0145	.0149
I Corn meal	.0022	.0017	.0072	.0064
J Farina	.0012	.0014	.0070	.0065

and since the final absorption is a function of the final concentration, one finds that smaller amounts of bromide are retained when smaller ratios of fumigant to product are employed.

Table I also shows that within the limits of experimental error the bromide residues are all inorganic except possibly for a small fraction in the case of wheat germ.

A number of prepared cereals were fumigated on a semicommercial scale to secure information on the amounts of bromide retained. Each sample was in its original restaurant-size or retail-size package and a full carton of the packages of each product was used in the test. All the cartons were fumigated simultaneously with 0.75 pound of methyl bromide for 18 hours in a 500-cubic-foot vault at 70°F. The dosage thus corresponded to 1.5 pounds per 1,000 cubic feet, or 4.05 pounds per 1,000 pounds of product (the load in the chamber was brought up by the addition of 135 pounds of shorts). The samples were analyzed after standing for one week in the original containers and the data obtained are shown in Table II. In only three cases were the retentions appreciably high, and full-scale commercial fumigation of these three would likely show lower retentions if a lower ratio of fumigant to product were used.

TABLE III

LABORATORY FUMIGATION OF WHOLE-WHEAT FLOUR

Hours aired	Total bromide	Inorganic bromide	Organic bromide
	%	%	%
0.5	.0299	.0178	.0121
4	.0244	.0183	.0061
24	.0213	.0197	.0016
48	.0210	.0199	.0011
96	.0202	.0196	.0006
168	.0199	.0197	.0002
Control, not fumigated	.0003	.0004	....

To show the states in which bromide occurs in fumigated wheat products at various times after fumigation, analyses were made of laboratory-fumigated flour, starch, and gluten. The conditions were purposely made conducive to the absorption of larger amounts of bromide in order to obtain greater variations. In Table III and Figure 1 are presented data found in the fumigation of whole-wheat flour with two pounds of methyl bromide per 1,000 cubic feet for 24 hours at 80°F. The dosage corresponds to a ratio of 330 pounds of methyl bromide to 1,000 pounds of product, since only 3 pounds of product were fumigated in a 500 cubic foot chamber.

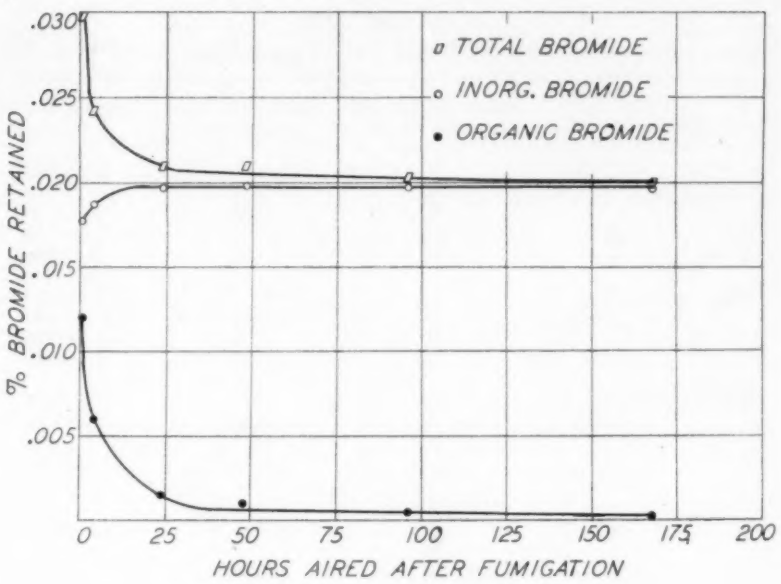

Fig. 1. Retention of bromide by whole-wheat flour.

TABLE IV

LABORATORY FUMIGATION OF WHEAT STARCH

Hours aired	Total bromide	Inorganic bromide	Organic bromide
	%	%	%
0.5	.0076	.0032	.0044
4	.0034	.0032	.0002
24	.0031	.0031	....
48	.0031	.0031	....
96	.0031	.0030	....
168	.0032	....	....
Control, not fumigated	.0006	.0006	....

TABLE V

LABORATORY FUMIGATION OF GLUTEN

Hours aired	Total bromide	Inorganic bromide	Organic bromide
	%	%	%
0.5	.0334	.0292	.0042
4	.0311	.0286	.0025
24	.0312	.0288	.0024
48	.0306	.0283	.0023
96	.0306	.0287	.0019
168	.0303	....	....
Control, not fumigated	.0016	.0018	....

TABLE VI

LABORATORY FUMIGATIONS OF WHEAT PRODUCTS AT DIFFERENT HUMIDITIES

Product	Relative humidity during fumigation	Hours aired after fumigation	Total bromide retained
	%		%
White flour	30	1	.0354
	30	168	.0230
	70	1	.0146
	70	168	.0144
Whole-wheat flour	30	1	.0296
	30	168	.0199
	70	1	.0178
	70	168	.0160
Wheat starch	30	1	.0261
	30	168	.0004
	70	1	.0025
	70	168	.0018
Gluten	30	1	.0418
	30	168	.0409
	70	1	.0407
	70	168	.0400

The results obtained with whole-wheat flour are similar to those reported for white flour (Shrader, Beshgetoor, and Stenger, 1941). Most of the absorbed methyl bromide escapes by volatilization and the remainder reacts to form inorganic bromide. The greater part of the bromide present half an hour after fumigation is already in the inorganic form.

Samples of wheat starch and gluten were simultaneously fumigated with 2.05 pounds of methyl bromide per 1,000 cubic feet for 12 hours in a drum at 88°F. The dosage of fumigant corresponded to 13.1 pounds per 1,000 pounds of product. The data are reported in Tables IV and V and Figures 2 and 3 and indicate that the gluten fraction of wheat retains more bromide than the starch. The opinion has occasionally been expressed that milled cereals retain appreciable quantities of bromide primarily because of their fine state of subdivision. The starch used in these experiments was ground as finely as the flour and much more finely than the gluten, yet it retained considerably less bromide. Evidently particle size is not the only determining factor in the absorption of bromide by cereals.

The effect of relative humidity on the absorption of methyl bromide during atmospheric fumigation has apparently not been studied previously. Unexplained differences in retentions of bromide obtained during summer and winter laboratory fumigations of flour led us to suspect that the humidity had some influence, so an experiment was performed to decide the question. Duplicate samples of each of several

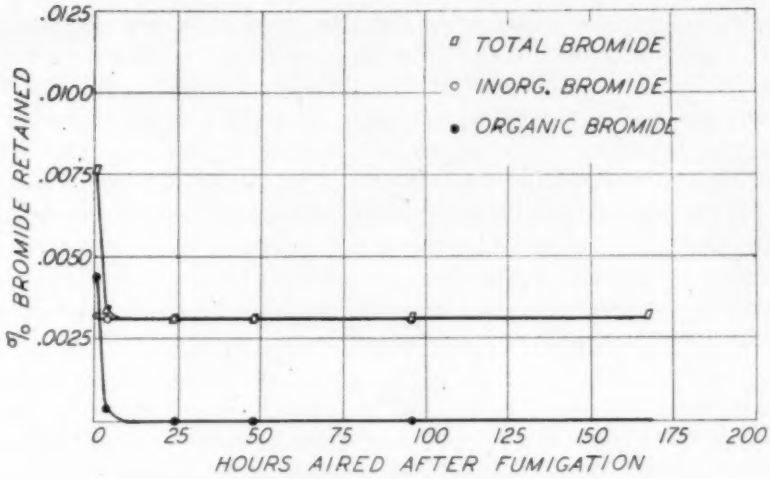

Fig. 2. Retention of bromide by wheat starch.

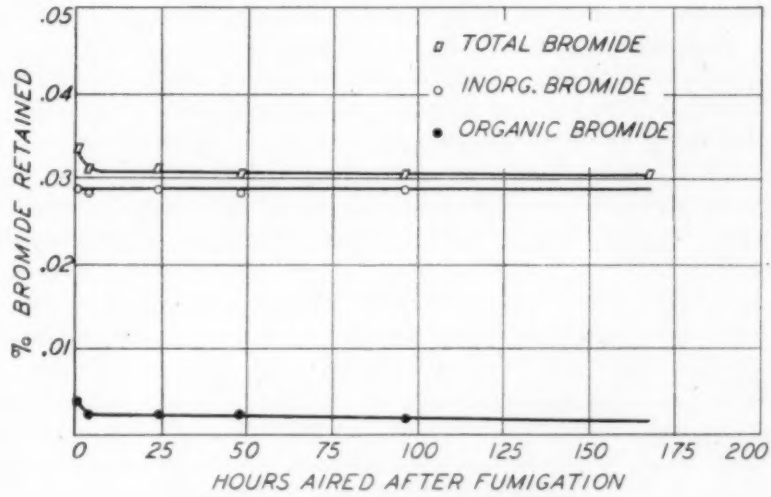

Fig. 3. Retention of bromide by wheat gluten.

wheat products were fumigated at two relative humidities in a 500-cubic-foot vault. The dosage in each case was two pounds of methyl bromide per 1,000 cubic feet, or 330 pounds per 1,000 pounds of product. Fumigation was continued for 24 hours at 80°F. The retentions, which naturally were higher than those obtained in commercial fumigations, are shown in Table IV.

Although the values appear rather confusing, they can be interpreted as being the result of two factors. First, water vapor is adsorbed preferentially to methyl bromide at the surface, so that with starch, for example, less methyl bromide is adsorbed at the higher

humidity. Second, adsorbed or absorbed methyl bromide hydrolyzes or reacts more rapidly in the presence of moisture. Thus the methyl bromide adsorbed on starch at 30% relative humidity escapes completely upon airing, whereas that taken up at 70% largely remains as inorganic bromide. With gluten, the bromide is taken up mainly by reaction and the surface displacement is almost wholly compensated by the increased reactivity under moist conditions. The effects noted with white and whole-wheat flour are intermediate between those observed with starch and gluten. It is of interest to realize that at low humidity more of the methyl bromide escapes by volatilization, while at high humidity more of it undergoes reaction.

Summary

It has been shown that the bromide retained by cereal products following fumigation with methyl bromide exists almost entirely in inorganic form after standing for a few days, and, furthermore, that the amounts retained are likely to be smaller in commercial fumigations than in laboratory experiments.

The retentions of bromide by different cereal products have been compared. Most commercial cereals retain only small amounts.

The influence of relative humidity on the retention of bromide by wheat products has been studied briefly. At higher humidities water displaces part of the methyl bromide from the surface, but the methyl bromide which is taken up reacts more rapidly to form inorganic bromide.

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EFFECT OF TEMPERATURE AND AGE ON MALT SYRUP

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(Read at the Annual Meeting, May 1941)

In the commercial handling of malt syrup it sometimes happens that syrup while in the package appears to undergo a marked change resembling fermentation. The customer usually observes this condition several hours after having opened the package and accepted what appeared to be a perfectly smooth, dark-surfaced syrup, free of any indication of foam or fermentation whatever. Within a few hours after opening, however, copious foaming may appear and proceed even to the extent of foaming out of the container. The customer naturally assumes from both the appearance and the odor that this supply of malt syrup is fermenting and rapidly becoming unfit for use.

Like many others we have made frequent investigations of this particular phenomenon and its accompanying effect on the syrup, and it is of interest to present related information from the literature as well as a discussion of certain data and material gathered by ourselves. We observe that the darkening in color, and the formation of carbon dioxide accompanying this condition, is a reaction fairly common to many sugars. It has long been known that certain sugars in combination with some amino acids undergo a definite chemical reaction.

Neuberg and Kobel (1926) showed that fructose and *dl*-alanine react at room temperature under neutral conditions. Other sugars, glucose and maltose, were likewise tested not only with alanine but also with aspartic and gluconic acids and with arginine. Very striking changes in rotation were found with aspartic and gluconic acids, especially in the case of fructose.

Euler, Brunius, and Josephson (1926) found that a condensation occurs between glycine and glucose with equilibrium in 30 to 40 hours. The reaction is reversible, the compound gradually undergoing cleavage when the alkaline solution is acidified. Condensation between glucose and alanine occurs in the same manner. Preliminary treatment of the glucose with NaOH does not alter the reaction. At pH 9.5 the condensation amounts to as much as 53% in 48 hours. The system glucose-glycine-NaOH at pH 9.3 decolorizes methylene blue, whereas glucose-NaOH alone, at the same pH, has no effect.

Maillard (1912) demonstrated that when glycocoll is warmed with 4 parts of glucose in 3 to 4 parts of water the liquid slowly assumes first a yellow, then a dark brown color, followed by foaming (CO_2).

The oxygen of the CO_2 comes from the carboxyl (CO_2H) group of the glycocoll. Assuming that this loss of CO_2 is accompanied by a union of the nitrogen with aldehyde carbon of the sugar, the glucose molecules (at least two in number) forming part of the new compounds must undergo dehydrations resulting in the appearance of double bonds or possibly rings. Various other amino acids react in the same way on glucose and similarly various sugars act in this way on glycocoll: xylose and arabinose immediately, fructose, galactose, glucose and mannose quite rapidly, lactose and maltose slowly, and sucrose not at all for several hours and then only very slowly.

Ambler (1929) states that the course of the reaction between glucose and amino acids depends entirely on conditions of temperature, concentration, and time. At high temperatures as much as 10 mols of glucose per mol of amino acid may be destroyed but lowering of the temperature reduces the effect. In this reaction compounds of progressive complexity of the general class of melanoidins are formed by dehydroxylation. The melanoidins greatly increase the tendency to froth and also increase the color of the products.

In the manufacture of sweetened condensed milk it was early noticed that with the use of glucose instead of sucrose the product had a marked tendency to darken in storage. Thus Haradine (1933) noted that the development of the brown color of sweetened condensed milk was attributable to the formation of humin due to reactions between protein or amino acids and sugars. The preheating temperature used in the manufacture of sweetened condensed milk has little effect upon the brown coloration, while temperature of storage had marked effects on the rate of its development. High storage temperature (*e.g.* 100°F) promoted rapid development of the brown color, while at storage temperature below 68° it formed slowly. The use of invert sugar syrup as a substitute for cane sugar in condensed milk caused a brown coloration. It is suggested that the inversion of sucrose is responsible for the brown coloration, this not being evident to the eye until 25% of the sucrose has been inverted. The critical temperature at which the brown coloration develops is 80°–90°F. The presence of iron salts was found to facilitate the development of color.

Our interest at first centered around the development of the foaming. In order to fully satisfy ourselves that, in the case of malt syrup, fermentation in no instance entered into the condition of such foaming syrup, innumerable samples were plated out at regular intervals. In no instance did we ever find that yeast had developed or that a usual yeast fermentation had taken place. Had yeast fermentation actually been the cause of the foam, the presence of

yeast cells in large numbers would certainly have been evident. Instead it will be shown that at temperatures well above pasteurization the formation of gas and foaming became progressively worse. In fact, we have often noticed that malt syrup either in cork-stoppered sample bottles or in other types of loosely stoppered containers never shows any foam nor any indication of sour odor or carbon dioxide whatever. In such containers fermentation could most easily take place were it possible. However, in malt syrup over 40° Bé it is doubtful that yeast growth could even exist.

In the experimental storage of samples of most malt syrups it was found that the syrup usually increased in color, especially if held at temperatures around 100°F. If the syrup was held in tightly sealed containers pressures of from a few ounces up to as high as 9 lbs were found to develop.

Since the literature refers to this relation between color and gas formation the following experiments were conducted. Various batches of different malt syrups were heated in different ways to study the effect of temperature on color, foam, protein and sugar.

Experiment No. 1: Three lots of 42° Bé syrup were heated in tubes at 165°F for 1 hour. A large amount of foam gathered on the surface of each sample.

Experiment No. 2: Malt syrup direct from process was heated in 600-ml beakers immersed in water baths in such a way that the syrup could be brought to the desired temperature in about 40 minutes, held at this temperature for 4-hour intervals, and then cooled and color determination made. It was then reheated for two more intervals of 4 hours each.

A great deal of foam formed on the surface of the 170°F beaker even to the extent of overflowing in 12 hours of heating. Foam formation was rather prominent in the 160°F beaker too, but only about a fifth as great as that of the 170°F sample. The 140°F and the 150°F beakers showed practically no foam in 12 hours' heating. The colors developed during this heating are given in the following table, the colors being on dilutions determined with the Lovibond Tintometer.

TABLE I

COLOR OF HEATED MALT SYRUP (42° BÉ SYRUP)

	4 hours' heat	8 hours' heat	12 hours' heat
Control	8	8	8
140°F	9	10	11
150°F	10	12	13
160°F	11	14	19
170°F	14	21	31

Protein determinations were made on these various samples of malt syrup, but showed no change even for the 170°F-heated malt syrup.

The degree of fermentation was also noted on these variously heated malt syrups by making a dilution of the syrup to a definite degree Balling (11.0). The degree of fermentation appeared to be

TABLE II

PROTEIN IN HEATED MALT SYRUPS (42° BÉ SYRUP)

	Protein
	%
Control	6.38
140°F	6.30
150°F	6.30
160°F	6.30
170°F	6.38

inversely affected by the higher heating, decreasing as the temperature is increased. The results are shown in Table III.

TABLE III

DEGREE OF FERMENTATION—MALT SYRUP HEATED FOR 12 HOURS

	Degree
	%
Control	81
140°F	76
150°F	71
160°F	69
170°F	64

Figure 1 shows the amount of foam present in the various beakers after the 12 hours of heating.

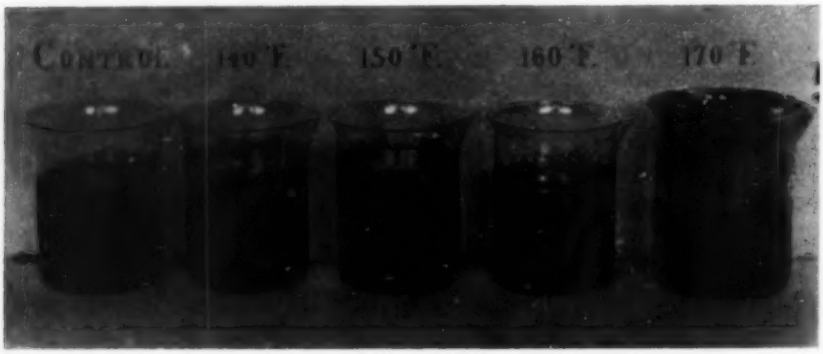

Fig. 1. Foam developed in 12 hours of heating.

Experiment No. 3: Light-density malt syrup from third effect vacuum pan (only 30° Bé) was heated in 600-ml beakers in water bath for 3 hours at the temperatures given below. Considerable

foam formed on the 190°F sample, less on the 180°F, and practically none on the others. Color, protein, and degree of fermentation are shown in Table IV.

TABLE IV

30° Bé Syrup from Third-Effect Pan Heated Three Hours

	Color	Protein	Available degree of fermentation
		%	%
Control	5.5	5.4	90
150°F	5.5	—	—
165°F	7.0	—	—
180°F	11.5	—	—
190°F	16.5	5.6	76

In these tests it is quite evident that color and gas develop together and become more rapid and profuse as the temperature increases. Little change took place in the proteins but about 15% of the sugar value was lost at the higher heating. These experiments indicate, therefore, that temperature plays a definite role in the darkening of malt syrup and gas formation.

Tests were then made of ordinary room temperature storage samples held for various periods. Color, acid, and diastase were obtained when the malt syrup was first made and when later examined at the interval shown in Table V.

TABLE V

Analysis of 42° Bé Malt Syrup after Storage at Room Temperature

(Original color about 60, acidity 0.45% to 0.60% and diastase 40° to 45° Lintner)

Lot no.	Time stored	Analysis after storage		
		Color	Acid	Diastase
	months		%	°*L*
1	2	95	0.50	46
2	3	160	0.55	44
3	4	185	0.61	45
4	5	210	0.68	43
5	6	270	0.73	42
6	7	350	0.76	43
7	8½	430	0.75	47
8	9	485	0.83	44
9	10	545	0.86	45
10	11	560	1.05	48

The syrups showed a marked increase in color, some increase in acidity, and little if any change in diastase. Other storage tests were made also over longer periods of time, as shown in Table VI.

TABLE VI

ADDITIONAL TESTS ON 42° BÉ MALT SYRUP STORED AT ROOM TEMPERATURE

Age	Acidity as lactic	Color 1" cell
months	%	
3	0.88	148
6	0.99	198
9	1.41	363
12	1.68	836
15	1.84	1352
18	1.85	1453
21	1.90	1503
24	1.96	1653

Conclusions

The above tests indicate that the color of malt syrup is a function of temperature and time, and that foaming results if the samples are hermetically sealed during long storage at room temperature or more rapidly at high temperatures even if open to the air. Acidity increases slowly but definitely and diastase appears to have been little affected for the times studied.

It would appear from these tests, therefore, that malt syrup should always be kept rather cool and never placed near a hot bake oven. And that if it does foam the foaming is not due to fermentation but to a reaction which appears to be accelerated by warm-temperature storage and by failure to provide proper venting for the carbon dioxide which apparently results and which will dissolve in the syrup. When such a tightly sealed container is opened the dissolved carbon dioxide soon begins to escape, causing the malt syrup to foam over. Cool storage and pin hole venting should be the remedy for this occasional behavior of malt syrup.

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REPORT OF THE MALT ANALYSIS STANDARDIZATION COMMITTEE

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(Read at the Annual Meeting, May 1941)

Upon the recommendation of last year's committee (1940) the ferricyanide method for the determination of diastatic power was adopted as a tentative method. The committee also recommended that certain phases of the method be studied further and that existing methods for the determination of alpha-amylase be submitted to preliminary investigation. This latter study is in progress, but has not been completed.

In the interest of unifying the methods recommended by our organization and those of the American Society of Brewing Chemists, a joint collaborative study was made of the A.S.B.C. (1940) and ferricyanide methods for the determination of diastatic power in malt. The primary purpose was to verify the conversion factor for the ferricyanide method as given by Anderson and Sallans (1937) which would give Lintner values that agree with those obtained by the A.S.B.C. method.

Samples of malt flour and soluble starch were sent to nineteen collaborators. On the basis of the method used for routine work, ten laboratories were selected to report on the ferricyanide method and nine on the A.S.B.C. method. The malt flour was to be used at four concentrations (10, 15, 20 and 25 g per 500 ml) to give diastatic-power values of approximately 75° to 190° Lintner. Duplicate determinations were to be made at each concentration on two days.

Nine collaborators submitted results obtained with the ferricyanide method and seven with the A.S.B.C. method. Since the primary aim of this study was to determine a conversion factor for the ferricyanide method, the means of the A.S.B.C. values were used as bases and these divided by the mean net ferricyanide titrations to give conversion factor for the different concentrations. The average value was 22.8 with a variation from 22.6 to 22.9. This is in excellent agreement with the factor suggested by Anderson and Sallans, which is 22.5 where 2 ml of infusion is used with a final volume of 250 ml. For convenience, it is recommended that a factor of 23 be used in calculation when the above conditions of diastasis are used.

The means for from two to four determinations for each collaborator at each concentration and for the two methods are given in Table I. Where the factor of 23 is used the agreement between the two methods is excellent and the variations within the two methods are of approxi-

TABLE I

DIASTATIC POWER IN DEGREES LINTNER (DRY BASIS) OF FOUR CONCENTRATIONS OF MALT FLOUR BY THE A.S.B.C. AND FERRICYANIDE METHODS—MEANS OF 2–4 DETERMINATIONS

A.S.B.C. METHOD

Collaborator	10 g	15 g	20 g	25 g
1	72	114	148	188
2	77	115	151	193
3	76	117	156	196
4	83*	119	159	203
5	72	108	137*	149*
6	72	106	143	180
7	73	117	153	185
Average	74	114	152	191

FERRICYANIDE METHOD

Collaborator	10 g	15 g	20 g	25 g
1	73	112	150	193
2	74	112	149	190
3	76	118	159	201
4	75	112	152	191
5	71	107	144	180
6	72	112	148	185
7	79	121	161	201
8	76	115	156	195
9	85*	126	164	205
Average	75	115	154	192

* Starred values not included in the averages.

mately the same magnitude. The average differences between duplicates and days, using the means for days and means for duplicates, were 1.1 for duplicates and 1.9 for days with the ferricyanide method. Similar values for the A.S.B.C. method were 1.3 and 1.8.

The committee recommends:

That a factor of 23 be used in calculating degrees Lintner from ferricyanide titration where the procedure of the A.S.B.C. method for diastasis is used.

That the results of the alpha-amylase study be published as soon as completed and that further study of the most promising methods be continued.

That close cooperation with the malt committee of the American Society of Brewing Chemists be continued on methods of mutual interest.

Acknowledgment

The chairman expresses his appreciation to the committee members and collaborators, whose willing cooperation made this report possible, and to R. I. Tenney and S. Jozsa of the American Society of Brewing Chemists for their cooperation and assistance in this study.

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CHANGES IN THE BARLEY KERNEL DURING MALTING: CHEMICAL COMPARISONS OF GERM AND DISTAL PORTIONS [1]

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(Read at the Annual Meeting, May 1941)

The chemical changes that occur during the malting of barley are of fundamental importance in the subsequent use of the malt. No attempt will be made here to review in detail the large number of studies which have been made of this subject. The changes in the activity of different enzymes during malting and kilning have been reviewed by Lüers (1936). In general, there is an activation or production of enzymes during the germination period, followed by a reduction during the kilning process. Ohlsson and Thörn (1938) followed the development of alpha-amylase during germination. This enzyme was practically absent until the third day of germination, when it increased rapidly, reaching its maximum in about five days.

These studies were made on the whole barley or malt kernels and gave an average picture of the changes within the whole kernel. Some early determinations were made by Brown and Morris, Windisch and Hasse, and Ling of the comparative amounts of diastase in the various sections of malt kernels. Windisch and Kolbach (1929) reviewed these and studied the total and cold-water-soluble nitrogen fractions in the germ and distal ends of barley and malt kernels after 15 days' germination. The total nitrogen was not greatly different in the two ends. In the barley there was somewhat more soluble nitrogen in the

[1] Cooperative investigations between the University of Wisconsin and the U. S. Department of Agriculture, Division of Cereal Crops and Diseases, Bureau of Plant Industry.

germ end than in the distal, and in the 15-day malt the soluble nitrogen in the germ end was from two to three times greater than in the distal end.

Histochemical or microchemical methods applied to microtome sections of material have been used to study barley and malt kernels by Linderstrom-Lang and others. Glick (1938) has applied this technique to studies on the ascorbic acid distribution in the developing embryo of the barley kernel, and gave his findings and a review of the findings of Linderstrom-Lang and Engel (1938) on the distribution of the amylase in the outer layers of the barley kernel. This technique provides valuable information but of necessity is applied to such small portions of the kernel that a complete picture of all the various changes taking place during germination requires many separate studies.

In the course of an investigation of malting methods there were available barleys and malts representing two to nine days of germination under various conditions. This material offered an excellent opportunity to obtain information on the chemical changes and enzyme distribution in the two halves of the kernels. A discussion of the changes in cellular structure with associated chemical changes during malting was given by Dickson (1940). The present paper represents a more detailed discussion of the chemical changes in the two portions of the kernels during germination.

Materials and Methods

Two varieties of barley, Oderbrucker (Wis. Ped. 5–1) and Wisconsin Barbless (Wis. Ped. 38) were malted at approximately 43% and 48% moisture at 16°C. Oderbrucker samples grown and malted in 1936 and 1937 and one sample of Wisconsin Barbless grown and malted in 1938 were used. Barley samples were steeped to the appropriate moisture contents and placed in the large malting unit at daily intervals for seven days. All were removed on the ninth day and given a uniform kilning treatment. This gave a series of malts grown from two to nine days under uniform conditions.

Samples of the barleys and the eight malts in each series were sectioned by hand into approximately equal halves. Samples of the sectioned portions and of the whole kernels were analyzed for moisture, total ash, total nitrogen, diastatic power, extract, and the various nitrogen fractions in the worts. Water extractions of the barleys and malts were made at 5°–7°C and total extract, total nitrogen, permanently soluble nitrogen, and formol nitrogen were determined on these extracts.

The methods of the American Society of Brewing Chemists (1937) were used for moisture and extract. Diastatic power was determined

by the ferricyanide method of Anderson and Sallans (1937). Total ash and nitrogen were determined by the methods of the Association of Official Agricultural Chemists (1935). Total wort nitrogen and cold-water-soluble nitrogen were determined on 25-ml aliquots. Permanently soluble nitrogen was determined on aliquots of the filtered sample after boiling for one hour under a reflux. Formol nitrogen was determined electrometrically with a glass electrode by adjusting a 25-ml aliquot to pH 9.0, adding formaldehyde, and titrating back to pH 9.0. Alpha-amylase determinations by the method of Blom and Bak (1938) were made on the Wisconsin Barbless series of 1938.

Presentation and Discussion of Data

Although the percentage of the germ and distal portions varied considerably from sample to sample as a result of variations in the cutting, the average values for quite a number of samples were 55% germ portion and 45% distal portion.

TABLE I

AVERAGE VALUES FOR TOTAL ASH AND TOTAL NITROGEN ON WHOLE KERNELS AND GERM AND DISTAL PORTIONS OF ODERBRUCKER AND WISCONSIN BARBLESS BARLEYS AND MALTS

	Whole-kernel barley	Distal end barley	Germ end barley	Mean of 2–9 day malts		
				Whole-kernel malt	Distal end malt	Germ end malt
	%	%	%	%	%	%
ODERBRUCKER						
Total ash	3.03	2.71	3.37	—	2.33	3.01
Total nitrogen	1.95	1.86	2.09	2.00	1.81	2.02
WISCONSIN BARBLESS						
Total ash	2.91	2.77	3.00	—	2.17	2.87
Total nitrogen	1.84	1.73	1.86	1.80	1.72	1.76

The large amount of data accumulated has been simplified in presentation by averaging values for the high and low moisture maltings of the 1936 and 1937 Oderbrucker samples. The data for Wisconsin Barbless are means of the high and low moisture malting series for one year, 1938. Where the effect of moisture is discussed, the values for each moisture are used. Most of the data are presented graphically to show the trends in the various constituents throughout the malting period.

Ash and Nitrogen

The percentages of total ash and nitrogen showed no consistent trend with malting time in either portion of the kernel so the data are presented in summary form in Table I, using the mean values for the eight malts. Ash was significantly higher in the germ ends of both barley and malt kernels than in the distal ends or in the whole kernels. Both ends of the barley kernels were higher in ash than the malt kernels, indicating an extraction of mineral materials during steeping and malting. The percentage loss from the distal portions was somewhat greater than from the germ portions.

The trends for nitrogen content in the two portions of the kernels were similar to those for ash, being slightly higher in the germ portion. This difference was less for Wisconsin Barbless than for Oderbrucker samples. The sectioned portions of the malt kernels were slightly lower in nitrogen than similar portions of the barley.

Diastatic Power

The data on diastatic power of the sectioned portions and the whole kernel of the two varieties are presented graphically in Figure 1. The characteristic difference between the two varieties in this factor is evident and accentuated in the germ portions of the kernels. The enzyme was activated more rapidly in Oderbrucker than in Wisconsin Barbless and a greater quantity was produced. The amylases

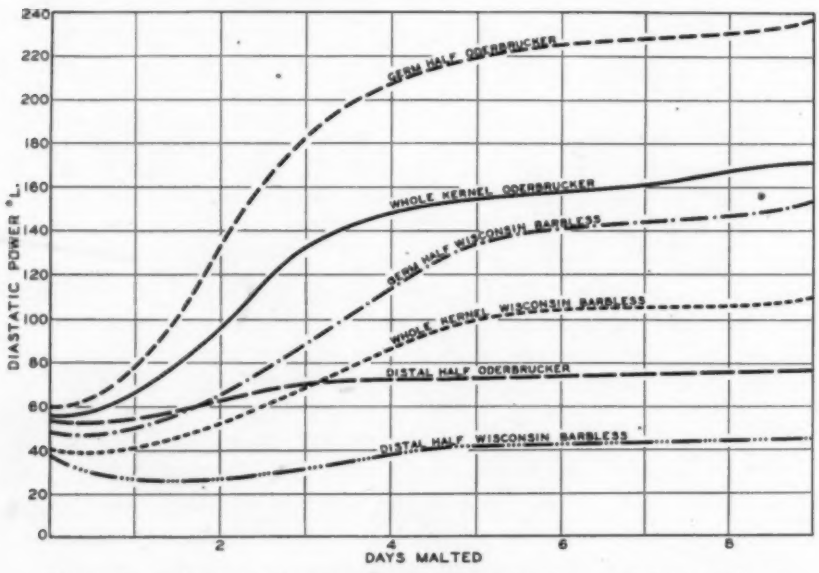

Fig. 1. The production of diastatic power in whole kernel and germ and distal portions of Oderbrucker and Wisconsin Barbless barley kernels during malting.

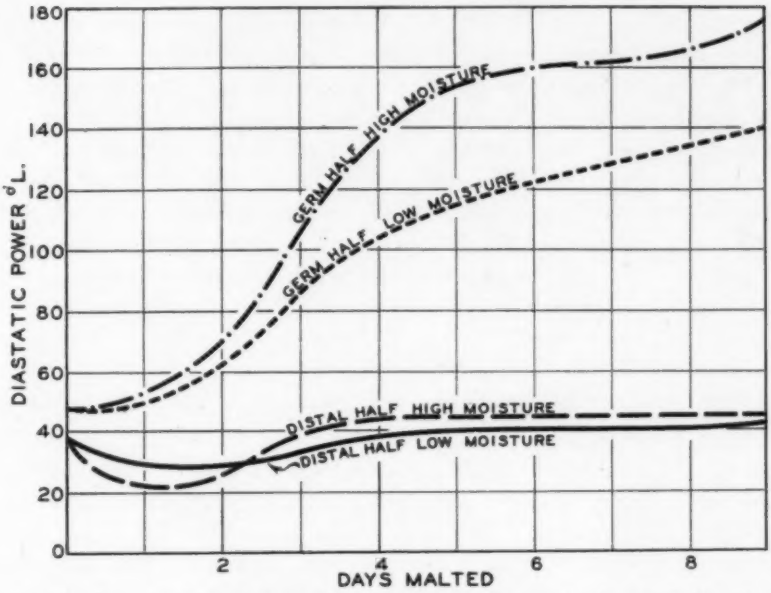

Fig. 2. The effect of moisture during malting on the diastatic power of germ and distal portions of malt kernels produced from Wisconsin Barbless barley.

measured by the diastatic power method, considered to be primarily beta-amylase, were activated or produced and retained principally in the germ half of the kernel. In the distal portion of the Oderbrucker kernels, the increase in diastatic power from the barley to the 9-day malt was 43%. In the germ half the increase was 293%. For Wisconsin Barbless the corresponding values were 18% for distal and 230% for germ halves.

These data indicate that the amylases were not activated or produced as rapidly or to the same extent in the distal portion as in the germ half of the kernel. Furthermore, if a movement of amylase from the site of greatest concentration during malting may be considered likely, the transfer into the distal end was not great. In 9-day malt from both varieties approximately 80% of the diastatic power of the whole kernel was found in the germ end and 20% in the distal end. In this connection, Linderstrom-Lang and Engel (1938) state that the amount by beta-amylase concentrated just within the aleurone layer constitutes approximately 20% of the total amylase of the kernel.

Diastatic power increased very rapidly in the germ portion and whole kernel until the fifth day. The increase after this was slight until the eighth or ninth day when a further slight increase took place as shown in Figure 1.

The influence of malting moisture on the diastatic power of the sectioned kernels of Wisconsin Barbless is shown in Figure 2. Since

the majority of the enzyme was produced in the germ end, the effect of moisture was much greater in this portion of the kernel. This was indicated by a more rapid rate of activation and an appreciably higher final value in the higher-moisture malting. The curve for the low-moisture malting shows a gradual rise throughout the nine days with the rate of activation decreasing appreciably after the fifth day. In contrast to this the high moisture curve is very similar to those shown for the germ portions and whole kernels in Figure 1. At 43% moisture, the amount of water may have been a limiting factor and thereby may have retarded the normal diastatic activation during germination.

Alpha-Amylase

Alpha-amylase by Blom and Bak's viscometric method was determined only on the Wisconsin Barbless sectioned kernels. The data showing the effect of moisture on this factor are shown graphically in Figure 3. The shapes of these curves are similar to those presented in Figure 2 for diastatic power. Perhaps the production of alpha-amylase was less influenced by moisture than was diastatic power. Here again the high moisture produced the flattening of the curve for the germ ends at the 6-, 7-, and 8-day malting periods.

The method used indicated practically no alpha-amylase in the distal ends, but a small amount in the germ ends of the barley kernels.

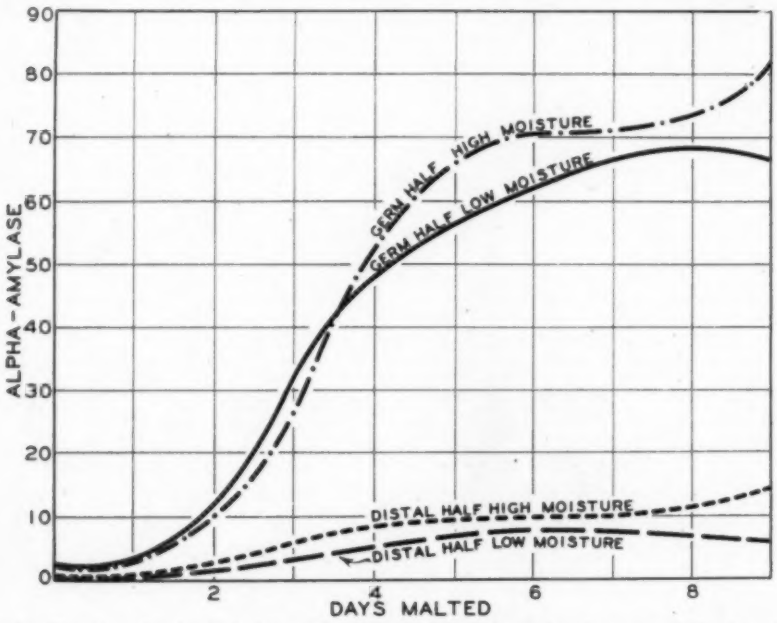

Fig. 3. The effect of moisture during malting on the alpha-amylase values for germ and distal portions of malt kernels produced from Wisconsin Barbless barley.

These values may have been caused in part by the presence of beta-amylase, since it has been shown to modify values obtained by this method. The small amounts of alpha-amylase in the barley were responsible for very large percentage increases from barley to 9-day malt. Using averages for the two moistures, the values for increase in alpha-amylase were 2,000% for the distal ends and 3,440% for the germ ends. This indicated that there was either a production of alpha-amylase in the distal end of the kernel or considerable diffusion from the germ end. However, at the end of 9 days of malting at high moisture approximately 85% of the alpha-amylase activity was in the germ half of the kernel. Therefore it appears that the distribution of alpha- and beta-amylase was approximately the same in the two halves of 9-day malt kernels. However, since there was practically no active alpha-amylase in the distal portion of the barley kernel, there was a much greater production of this component than of the beta component in the distal half during malting.

Soluble Nitrogen Fractions

The total cold-water-soluble nitrogen should be a measure of the amount of hydrolysis of proteins during malting. The extraction was made at a temperature of 5°–7°C in order to minimize the activity of the enzyme during extraction. On the other hand wort nitrogen is a measure of the amounts of hydrolysis occurring during malting and laboratory mashing. Wort nitrogen appears to be a fairly reliable indicator of proteolytic activity. In a series of malts representing different varieties furnished by this laboratory and reported upon by Koch, Nelson, and Ehrnst (1939), there was a very high correlation between the proteolytic power determined by their method and both the wort nitrogen and wort formol nitrogen. The data for total cold-water-soluble nitrogen and wort nitrogen, both calculated as percentage of barley or malt nitrogen, are presented graphically in Figure 4.

These curves show the trends in the nitrogen fractions in the two portions of the kernel and the whole kernels of the two varieties during malting. A marked similarity in the shapes of the curves for the two fractions is evident. As was true of the amylases, the activity of the proteolytic enzymes was consistently greater for Oderbrucker than for Wisconsin Barbless. The difference between varieties was again greater in the germ than in the distal portions, although this was not so marked as with the amylases. In fact, when expressed as percentage of the amount of nitrogen present in the two portions, the difference between the varieties was about equal in the germ and distal halves.

It is apparent from the graphs that there was much greater enzymatic activity in the germ than in the distal portion. Considering the cold-water-soluble nitrogen, where it was possible to obtain this value on the barley sections, there was a 95% increase in the distal portion of the Oderbrucker from barley to 9-day malt. The corresponding increase for the germ portion was 176%. Similar values for Wisconsin Barbless were 114% for distal portions and 166% for germ

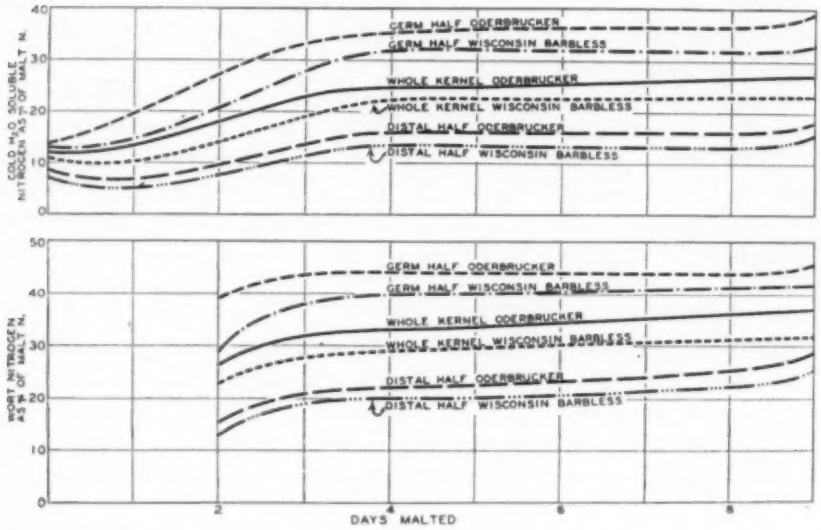
COLD H2O SOLUBLE NITROGEN AS % OF MALT N.
GERM HALF ODERBRUCKER
GERM HALF WISCONSIN BARBLESS
WHOLE KERNEL ODERBRUCKER
WHOLE KERNEL WISCONSIN BARBLESS
DISTAL HALF ODERBRUCKER
DISTAL HALF WISCONSIN BARBLESS
WORT NITROGEN AS % OF MALT N.
GERM HALF ODERBRUCKER
GERM HALF WISCONSIN BARBLESS
WHOLE KERNEL ODERBRUCKER
WHOLE KERNEL WISCONSIN BARBLESS
DISTAL HALF ODERBRUCKER
DISTAL HALF WISCONSIN BARBLESS
DAYS MALTED

Fig. 4. The production of wort nitrogen and cold-water-soluble nitrogen in whole kernel and germ and distal portions of Oderbrucker and Wisconsin Barbless barley kernels during malting.

portions. The value for the germ half represented a doubling or less of the percentage increase in the distal half. In the case of the diastatic power, the percentage increase in the germ portion was 7 and 13 times that in the distal portion in Oderbrucker and Wisconsin Barbless, respectively. This indicates a much greater production or activation of the proteolytic enzymes in the distal ends of the kernels, or a fairly rapid transfer of these enzymes throughout the kernel during malting. Histological evidence, as presented by Dickson (1940), indicated that probably the latter was the case, since the increase in cold-water-soluble nitrogen was associated with the removal of the starch embedding matrix of the endosperm. The removal of the embedding materials started adjacent to the scutellum and progressed toward the distal end of the kernel as malting continued.

The data for cold-water-soluble nitrogen and wort nitrogen are presented in a different manner in Figure 5. Considering the cold-water-soluble-nitrogen as that solubilized during malting, the difference between the wort nitrogen and cold-water-soluble nitrogen represented

the additional amount of nitrogen made soluble during laboratory mashing. The cold-water-soluble nitrogen in the germ and distal portions of the barleys was subtracted from both values for the malts. The shaded portion of the diagram then represents the amount of nitrogen made soluble during malting, while the clear area under the upper curve represents the amount made soluble during laboratory mashing.

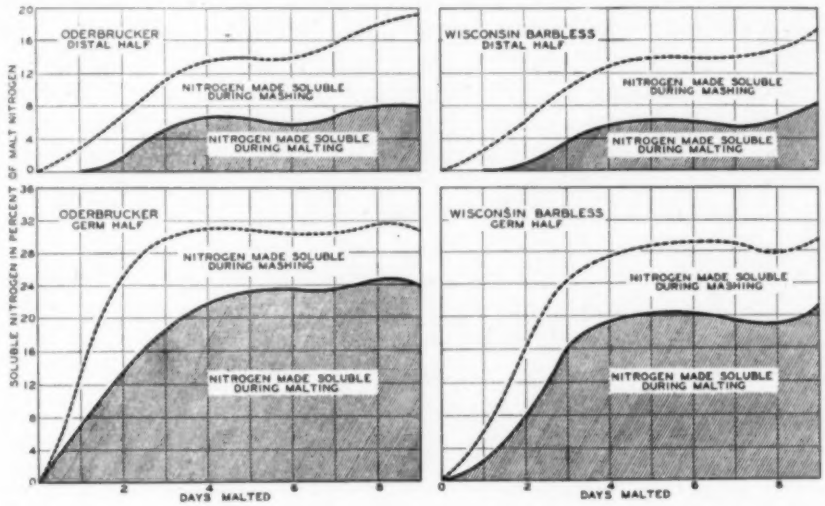
ODERBRUCKER DISTAL HALF
NITROGEN MADE SOLUBLE DURING MASHING
NITROGEN MADE SOLUBLE DURING MALTING
WISCONSIN BARBLESS DISTAL HALF
NITROGEN MADE SOLUBLE DURING MASHING
NITROGEN MADE SOLUBLE DURING MALTING
ODERBRUCKER GERM HALF
NITROGEN MADE SOLUBLE DURING MASHING
NITROGEN MADE SOLUBLE DURING MALTING
WISCONSIN BARBLESS GERM HALF
NITROGEN MADE SOLUBLE DURING MASHING
NITROGEN MADE SOLUBLE DURING MALTING
SOLUBLE NITROGEN IN PERCENT OF MALT NITROGEN
DAYS MALTED
DAYS MALTED

Fig. 5. Comparative amounts of total nitrogen made soluble during malting and mashing in germ and distal portions of Oderbrucker and Wisconsin Barbless malts.

In the distal portion of the Oderbrucker 9-day malt, 41% of the total amount of soluble nitrogen was made soluble during malting, leaving 59% made soluble during mashing. This ratio was practically the same for the 6-day malt. In the germ portion, even at 6 days, 80% of the nitrogen was made soluble in malting and only 20% was solubilized during mashing. This ratio was unchanged at 9 days. Wisconsin Barbless presented a somewhat different picture. In the germ end, the ratios were about the same in 6- and 9-day malts with 70% of the total soluble nitrogen made soluble during malting. In the distal ends at 6 days 42% of the nitrogen was solubilized during malting and at 9 days this value had increased to 47%. Wisconsin Barbless malts contained a smaller amount of soluble nitrogen than Oderbrucker and a smaller percentage of that nitrogen was made soluble during malting. Also in the distal end the nitrogen was made soluble during malting at a slower rate than in Oderbrucker, but eventually a greater percentage of the total was solubilized. This was further evidence of the greater enzymatic activity of Oderbrucker malt.

Permanently soluble and formol nitrogen were determined in the wort and in the cold-water extract. With possibly one exception, the correlation between these two factors and the total soluble nitrogen in the wort and in cold water extract was very high within any one kernel portion of a variety. Therefore, the data for these factors will not be presented graphically, as they should show the same relationships as wort nitrogen and cold-water-soluble nitrogen. Permanently soluble nitrogen indicated certain differences between the germ and distal portions of the malt kernels. In the laboratory worts, the permanently soluble nitrogen made up approximately 95% of the total wort nitrogen in both the germ and distal halves of the two varieties. This ratio remained fairly constant during the malting period. In the cold-water extract of the distal portions from the two varieties, the permanently soluble nitrogen constituted approximately 80% of the total cold-water-soluble nitrogen, and did not vary greatly from 2- to 9-day malts. In the germ portion, the permanently soluble cold-water nitrogen constituted a decreasingly smaller percentage of the total as malting progressed from 2 to 9 days. The average values for the 8 malts were 70% for Oderbrucker and 66% for Wisconsin Barbless. Considering the cold-water extract as an indication of the changes during malting, a smaller amount of the total nitrogen was permanently soluble in the germ end than in the distal portion, and this ratio became decreasingly less as malting progressed.

Laboratory and Cold-Water Extract

The data for laboratory extract on the germ and distal portions of the malt kernels and total cold-water extract on the barley and malt kernels are presented graphically in Figure 6. The presentation is similar to that used for the nitrogen fractions, the cold-water-extract values of the barley halves being subtracted from the malt values. The two fractions can then be looked upon as those made soluble in malting and mashing, respectively, but the values in the graph must be increased by approximately 8% to give the true extract values.

The distal halves of Oderbrucker kernels showed a higher laboratory extract value than those of Wisconsin Barbless, and the earlier activation of the amylases was indicated by the proportionately higher value for the 2-day malt. All of the values for the distal halves of 2- and 3-day malts are low because of insufficient enzyme to bring about conversion in one hour. The extract of the germ portions of Wisconsin Barbless malts was somewhat higher than for Oderbrucker, although the extract on the whole kernels was higher in the Oderbrucker.

In contrast to the soluble nitrogen fractions, the amount of extract made soluble in malting was a much smaller percentage of the total

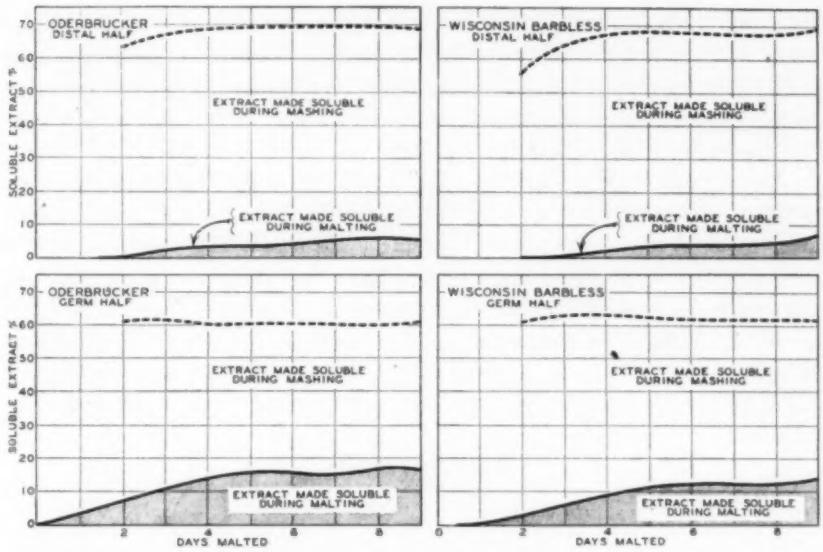

Fig. 6. Comparative amounts of extract made soluble during malting and mashing in germ and distal portions of Oderbrucker and Wisconsin Barbless malts.

extract even in the germ portion. At the end of 9 days of malting 28% and 22% of the total extract were made soluble during malting in the germ portions of Oderbrucker and Wisconsin Barbless, respectively. Corresponding values for soluble nitrogen were 80% and 70%. The percentage of total extract made soluble in the distal portions of the two varieties did not differ greatly after 6 days. Approximately 6% was soluble at 6 days and this value increased to 9% at 9 days of malting. This appears to be further evidence that the proteolytic enzymes are distributed throughout the kernel much more completely than the amylases. In the case of the latter most of the hydrolysis of the starch takes place during mashing, after the material has been ground and the enzyme made available. Microscopical evidence presented by Dickson (1940) indicated very little decomposition of the starch grains even in 9-day malt.

Summary

The kernels of barleys and malts representing 2 to 9 days' germination of these barleys were sectioned into approximately equal halves. These were submitted to analysis with particular emphasis on the enzymatic changes taking place during germination. The data are presented graphically showing trends for the various factors during malting.

Total ash and nitrogen were reduced by steeping but did not vary appreciably during malting. Both were higher in the germ portion,

the difference being greater for ash than for nitrogen. Diastatic-power determinations indicated that the majority of the beta-amylase production or activation was in the germ half of the kernel and remains there. Alpha-amylase showed somewhat wider distribution throughout the kernel than the beta-amylase.

The proteolytic enzymes, measured by cold water and wort nitrogen, appeared to be distributed much more widely throughout the kernel than either of the amylases. From 70% to 80% of the total protein hydrolysis took place during malting in the germ half and only 20% to 30% during mashing. Carbohydrate hydrolysis during malting was comparatively low, in the germ end, attaining 20% to 25% of the total. This is interpreted as indicating contrasting activation and distribution of the two groups of enzymes during malting.

Acknowledgments

Acknowledgment is given to J. G. Dickson and H. L. Shands for production of the materials used in this investigation and for advice and assistance in the preparation of the manuscript. Eugene Herrling is given credit for the preparation of illustrative material. Assistance in the preparation of these materials was furnished by the personnel of the Works Projects Administration Project No. 65–1–53–2349.

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COLLABORATIVE STUDY OF THE AYRE AND ANDERSON METHOD FOR THE DETERMINATION OF PROTEOLYTIC ACTIVITY [1]

F. C. HILDEBRAND

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1941)

A previous study by the author (1940) compared the Landis and Frey rate of gelation and the trichloracetic acid methods for the determination of proteolytic activity. While this study was based entirely on malted wheat flours as the experimental material, it was believed that results obtained could be applied equally well to other cereal products. In the study cited above, it was found that both methods give essentially similar results and that they have almost exactly the same experimental error and ability to differentiate between samples. The trichloracetic acid technique, as given by Ayre and Anderson (1939), was preferred because of its greater simplicity and convenience. In this procedure, duplicate samples of the material to be tested are autolytically digested at 45°C for one- and three-hour periods. Following digestion, trichloracetic acid is added to precipitate residual protein, and after filtration, determinations of soluble, *i.e.* nonprotein, nitrogen are made on aliquots of the respective filtrates. Proteolytic activity is calculated from the increase in nonprotein nitrogen from one to three hours' digestion.

In order to test the applicability of this method to materials other than malted wheat flour and to obtain an estimate of its reliability when applied in different laboratories, a collaborative study of the utility of the method was organized.

Experimental

Ten collaborators [2] tested a total of five samples. Included in the samples were a patent and a clear flour milled from a southwestern winter wheat; two samples of barley malt, one of high and one of low diastatic activity; and a sample of commercially produced malted wheat flour. The samples of barley malt were ground to pass a 100-mesh screen, and all samples were blended thoroughly by mechanical means. Subsamples were taken and immediately sealed and shipped to the several collaborators. The collaborators analyzed in duplicate the five

[1] Paper No. 28, Journal Series, General Mills, Inc., Research Laboratories. Subcommittee report, 1940–41 Committee on Methods of Analysis.

[2] The author is indebted to W. G. Artis, G. S. Bratton, George Burkert, B. A. Burkhart, W. F. Geddes, R. H. Harris, Quick Landis, Stephen Laufer, L. E. Leatherock, and W. O. S. Meredith for their generous collaboration in this project.

samples submitted with the results shown in Table I. In order to facilitate comparisons, an analysis of the data has been made, as shown in Tables II, III, and IV.

TABLE I

COLLABORATIVE DATA ON MEASUREMENT OF PROTEOLYTIC ACTIVITY BY THE AYRE AND ANDERSON METHOD

Sample	Collaborator										
	A	B	C	D	E	F	G	H	J	K	Av.
1	82	77	137	96	109	115	103	92	71	114	100.0
	84	69	153	92	119	94			94	105	
2	221	197	127	257	285	247	310	249	242	198	230.4
	231	160	122	255	234	244			271	198	
3	254	221	165	294	299	297	310	292	270	225	271.9
	264	360	159	300	321	300			276	229	
4	65	87	67	63	49	39	140	0	35	53	49.0
	55	33	72	66	35	39			32	51	
5	264	180	162	261	253	263	211	225	262	242	233.6
	274	164	172	263	279	274			242	246	
Av. all samples	179.4	154.8	133.6	194.7	198.3	191.2	214.8	171.6	179.5	166.1	177.0

Figures represent mg nonprotein N per 100-g sample.

TABLE II

SUMMARY OF COLLABORATIVE DATA—EIGHT COLLABORATORS

VARIATION BETWEEN SAMPLES

Sample No.	1	2	3	4	5
Mean activity, as mg NPN/100 g	107.4	232.6	282.3	56.0	253.4

$F^1 = 56.9$† 1% pt = 4.57

VARIATION BETWEEN COLLABORATORS

Collaborator	A	B	C	D	E	F	J	K
Mean activity, as mg NPN/100 g	179.4	154.8	133.6	194.7	198.3	191.2	179.5	166.1

$F^2 = 12.5$† 1% pt = 3.12

Interaction $S \times C$, $F^2 = 6.30$† 1% pt = 2.24
Standard error of single determination = 19.8 mg.

F^1 = interaction variance taken as error.
F^2 = duplicate variance taken as error.
† Highly significant.

TABLE III

SUMMARY OF COLLABORATIVE DATA—FIVE COLLABORATORS

VARIATION BETWEEN SAMPLES					
Sample No.	1	2	3	4	5
Mean activity, as mg NPN/100 g	95.6	248.1	287.5	47.8	263.5
$F^1 = 357.2$†		1% pt = 4.77			
VARIATION BETWEEN COLLABORATORS					
Collaborator	A	D	E	F	J
Mean activity, as mg NPN/100 g	179.4	194.7	198.3	191.2	179.5
$F^2 = 5.51$†		1% pt = 4.18			

Interaction $S \times C$, $F^2 = 2.40$* 5% pt = 2.06 1% pt = 2.81
Standard error of single determination = 11.8 mg.

F^1 = interaction variance taken as error.
F^2 = duplicate variance taken as error.
* Significant.
† Highly significant.

TABLE IV

COMPARISON OF STATISTICAL CONSTANTS—EIGHT AND FIVE COLLABORATORS

Variation due to	Eight collaborators	Five collaborators
	F	*F*
Between samples	56.9†	357.2†
Between collaborators	12.5†	5.51†
Interaction $S \times C$	6.30†	2.40*
Standard error of single determination	19.8 mg	11.8 mg

* Significant.
† Highly significant.

Discussion

In analyzing the data, the values submitted by collaborators G and H have been omitted since these individuals did not submit duplicate results. It will be seen that the method is adequate to distinguish between materials with as wide a range of proteolytic activity as was exhibited by the samples submitted. In addition, while the precision of the method is not as high as that of well standardized and common cereal chemical techniques, the results obtained by all collaborators compare favorably with data previously given by Ayre and Anderson. These authors report obtaining a precision of 18.6 units (standard error of a single determina-

tion) compared with the value of 19.8 obtained in the present study. It is indeed surprising that as high a level of precision was obtained in view of the fact that approximately half of the collaborators had not had previous experience with this technique.

Despite this relatively high precision, there are significant differences between laboratories and a significant tendency for the various collaborators to obtain relatively different results with the several samples. In general, however, the data indicate that this technique can be used by different laboratories with the expectation of obtaining fairly concordant results. If the method is to be applied extensively, it might be desirable to expend further effort to determine the cause or causes of the observed inter-laboratory variability. It has been suggested that one possible source of difficulty may lie in the use of containers of different wall thickness, which would cause different rates of heat transmission to the digestion mixtures.

As a matter of interest, the data shown in Table I were re-analyzed, omitting the data of the three collaborators B, C, and K. The results of this second analysis are shown in Table III, and the two are compared in Table IV. It will be seen that the inclusion of data from only five collaborators materially improves the differentiation between samples and the apparent precision of the method, while at the same time reducing the variability between collaborators and also reducing the magnitude of the interaction variation. These comparisons indicate that there is a distinct possibility of obtaining satisfactory results by the use of the Ayre and Anderson technique. It is believed that the improvement in results shown in the second analysis is due to the greater experience of the five collaborators with this method although it is possible that the difference is purely fortuitous.

Summary

The Ayre and Anderson trichloracetic acid precipitation method for the determination of proteolytic activity has been studied collaboratively. Ten collaborators have applied the method to samples of a patent and a second clear flour, two of barley malt, and a malted wheat flour.

The level of precision attained appears to be satisfactory. The standard error of a single determination was found to be 19.8 mg nitrogen per 100 g, as against a comparable value of 18.6 mg given by Ayre and Anderson (1939). Significant tendencies were found, however, for different laboratories to obtain different results and for the several laboratories to obtain results varying with the different samples.

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SOME PRELIMINARY STUDIES PERTAINING TO THE OPERATION AND USE OF THE BUHLER MILL

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(Read at the Annual Meeting, May 1941)

We have assumed that the primary purpose of the Buhler mill is to produce a flour from any particular sample of wheat, so that by means of interpretation and correlation the milling, blending, and baking values of the wheat may be determined prior to its being milled on the commercial unit. An exact duplication of the commercial flour with respect to flour yield, color, granulation, ash content, baking quality, etc. did not appear to be possible although it did seem probable that sufficient characteristics could be correlated to give the essential information. Since our main interest was to secure a knowledge of flour quality, we attempted to match the straight-grade experimental flour with the commercial flour of a similar extraction. At the same time, correlations with respect to milling characteristics were also desirable.

It must be pointed out that the scope of our experiments has been limited to our local conditions and to the use of Canadian spring wheats. These wheats were drawn from unload shipments at our own elevator, none of which therefore was of "pure variety" but consisted of the statutory grades from No. 1 Manitoba Northern to No. 4 Manitoba Northern, special grades (rusted or otherwise shrunken), and Garnet (a very vitreous variety). The data relative to the correlations between the Buhler and a commercial unit were obtained by using a 1500-barrel mill.

Buhler Mill Flow

The flow of our Buhler mill is similar to that outlined by J. E. Anderson [1] with the exception that a 10XX flouring silk is used on the first break section instead of 9XX and a 46 GG scalp replaced the 40 GG on the first reduction section.

[1] J. E. Anderson: Comparison of experimental and commercial milling results, The Northwestern Miller (Production No.), August 10, 1938, pp. 48–53.

Mechanical features of the mill: There are three break sections and three reduction sections. The three breaks are built on one pair of rolls, the first break having 16 corrugations per inch, the second 21 corrugations, and the third 26 corrugations.

The three reductions are likewise built on one pair of rolls which are smooth surfaced. The speed of the fast roll is 540 rpm and the differential is 2 : 1.

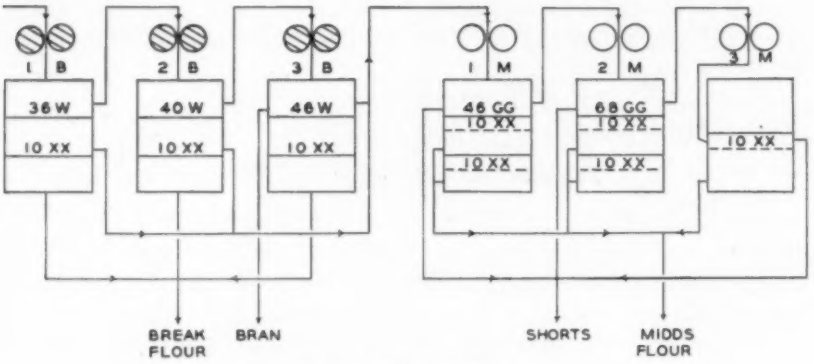

Fig. 1. Buhler mill-flow.

Wheat Conditioning or Temper

In order that flour yields from wheats of varying moisture content may be compared, a correction to a 13.5% moisture basis is made on the original sample of wheat when weighed into the mill.

The amount of moisture contained by the wheat and the distribution of this moisture at the time of grinding appear to have a definite effect on the milling conditions. The following method of tempering was adopted with good results. The wheat is scoured "as received" and then tempered to 16.0% moisture and allowed to stand for 24 hours. The added water may be applied with an atomizer or pipette and the sample thoroughly shaken up in a tin container. The sample is then scoured a second time before grinding with no further temper. To simplify the procedure, 2,100 g of wheat is weighed out on the basis of 16% moisture. An original sample of 2,100 g is used to allow for the loss in scouring, and for a moisture sample to be taken.

Example:

Moisture content of wheat "as received" = 10%. Weight of wheat at 10% moisture on basis of 2,100 g at 16% = $2{,}100 \times \frac{100 - 16}{100 - 10}$ = 1,960 g. Water (ml) required to raise 1,960 g wheat to 16% moisture = 2,100 − 1,960 = 140.

After the second scour 2,060 g of wheat is weighed out $\left(2{,}000 \times \frac{100 - 13.5}{100 - 16}\right)$ and the yield is computed on 2,000 g at 13.5% moisture basis. It will be noted that no second temper immediately prior to milling is employed. This is explained by the fact that the clean-up of the bran is visibly affected. It was found that a second temper immediately prior to milling, sufficient to raise the moisture content of the wheat by 1%, increased the yield of bran by as much as 1½% and decreased the yield of break flour by a like amount, without significantly affecting the ash content of the flour.

In milling the more vitreous wheats, a temper of 17% to 17.5% moisture is used. An "invisible" loss to the extent of 0.5% moisture usually occurs during the 24-hour temper. Milling tests of a commercially tempered wheat and of the same wheat experimentally tempered failed to show any significant difference in results.

It is our conclusion that for successful milling on the Buhler, the wheat must be thoroughly mellowed by adding sufficient moisture in the first temper. A second temper immediately prior to milling appears to cause a stickiness in the bran and prevents a satisfactory clean-up.

Grinding

Under the commercial system, wheats of varying weight per bushel or size of kernels would require a variance of roll settings on the first break. With the Buhler, however, the first-break roll is set closely enough to thoroughly break up the wheat kernel. Very little adjustment of roll setting, therefore, is necessary when one is grinding either a 60-lb or a 56-lb wheat. In order to obtain a knowledge of the operation of the Buhler, the character of the stocks and the type of flours that can be obtained, numerous samples of wheat of varying condition and weight per bushel were milled under a series of conditions. These included variations in temper, roll settings, and rate of feed. Hand sifting and chemical analysis of all the mill stocks gave an approximate idea of the percentage separations. These results are shown in Table I. As a rough check on the accuracy of Table I, Table II shows the comparative percentage separations by the milling test and by the hand-sifting method.

Since there is no classification of middlings, and because of the short reduction system, a partial reduction of the middlings must be accomplished on the break rolls. Approximately 70% of the first-break stock, a considerable amount of which is coarse middlings, is scalped over to the second break. Some of these middlings are reduced and 55% of the total break flour is obtained from the second

TABLE I

BUHLER MILLING DATA—HAND-SIFTED STOCKS

Flour	Roll	First break	Separation			Moisture	Ash	Protein
	%	%		%	%	%	%	%
1st break	2000	100	Over 36W	1400	70	14.8	1.86	15.0
			Over 10XX	480	24	14.2	0.56	12.4
			Thru 10XX	120	6	14.1	0.47	14.9
2nd break	1400	70	Over 40W	490	35	13.9	3.50	16.8
			Over 10XX	700	50	14.4	0.54	12.7
			Thru 10XX	210	15	14.7	0.45	16.3
3rd break	490	24.5	Over 46W	343	70	13.7	4.80	17.1
			Over 10XX	98	20	13.5	1.70	15.4
			Thru 10XX	49	10	13.6	0.76	19.2
1st midds	1278	63.9	Over 46GG	102	8	14.0	1.86	13.6
			Over 10XX	396	31	12.9	1.09	13.3
			Thru 10XX	780	61	13.9	0.40	12.8
2nd midds	396	19.8	Over 68GG	59	15	12.6	3.33	16.0
			Over 10XX	111	28	12.5	1.78	14.7
			Thru 10XX	226	57	12.6	0.52	12.5
3rd midds	111	5.5	Over 10XX	48	43	12.5	2.16	14.8
			Thru 10XX	63	57	11.4	0.82	13.5
Break	—	—	—	379	18.95	13.9	0.52	16.6
Midds	—	—	—	1069	53.45	13.0	0.465	12.7
Straight grade	—	—	—	1448	72.40	13.4	0.48	13.6
Shorts	—	—	—	209	10.45	11.9	2.60	15.0
Bran	—	—	—	343	17.15	13.7	4.80	17.1
Yield	—	—	—	—	72.40 = 4 bu, 30 lbs, 11 oz per barrel (Imperial measure)			

TABLE II

COMPARATIVE PERCENTAGE SEPARATIONS BY THE MILLING TEST AND BY THE HAND-SIFTING METHOD

Roll settings:	1st break	10.0	1st reduction	6.6
	3rd break	3.8	3rd reduction	3.2
Grinding time:	20 minutes			
Feed gate:	5 (scale reading)			
Size of sample:	2,000 grams			

	Milling test	Hand-sifted stocks
	%	%
Break flour	17.15	18.95
Midds flour	54.75	53.45
Total flour	71.90	72.40
Shorts	7.00	8.45
Bran	20.75	19.15
Total feed	27.75	27.60
Total products	99.65	100.00
Loss	0.35	0.00
Yield	4.31.12	4.30.11

break. The third-break stock is mainly bran (3.5% ash) and 70% of this is tailed over to bran. Although the ash content of the third-break flour is rather high, it is not thought necessary to exclude it from the total flour.

The first-middlings stock, being made up of the second separation of all three breaks, contains middlings of varying size, the largest of which are the throughs of 36W and the smallest the tailovers of 10XX silk. This stock is made up approximately as in Table III.

TABLE III

COMPOSITION OF STOCK TO FIRST MIDDLINGS ROLL

Stock	Percent of midds stock	Ash	Protein
	%	%	%
1st midds	100	0.65	13.0
1st break over 10XX	37½	0.56	12.4
2nd break over 10XX	55	0.54	12.7
3rd break over 10XX	7½	1.70	15.4

Most of the middlings flour must be made on the first reduction section so that the tail of the mill is not overloaded. This requires very close grinding and results in some of the larger-sized middlings being flaked and scalped off to shorts by the 46 GG. However, there appears to be no alternative, for if the 46 GG is removed so that the flaked middlings may be reground on the second middlings roll, some of the bran particles which are present in the same stock are almost sure to appear in the second middlings flour. On the other hand, a finer scalp on first break tends to upset the balance of the mill. The

TABLE IV

ANALYSIS OF BREAK FLOUR

Flour	Percent of total flour	Percent of break flour	Ash	Protein
	%	%	%	%
Break	26	100	0.52	16.6
1st break	8.3	32	0.47	14.9
2nd break	14.5	55	0.45	16.3
3rd break	3.3	13	0.76	19.2

ANALYSIS OF MIDDLINGS FLOUR

Flour	Percent of total flour	Percent of midds flour	Ash	Protein
	%	%	%	%
Midds	74	100	0.46	16.6
1st midds	53.8	73	0.40	12.8
2nd midds	15.6	21	0.52	12.5
3rd midds	4.5	6	0.82	13.5

question has arisen as to whether a finely "scratched" or "figured" roll on first reduction would help offset the flaking of the larger middlings. The roll settings as shown in Table II were found to be most suitable for the reduction of the various stocks. Little adjustment is considered necessary for grinding wheats of different weight per bushel, apart from reducing the rate of feed for lighter-weight wheats and *vice versa*. The percentage of separation of the flours is shown in Table IV.

TABLE V

REPLICATE MILLINGS

Wheat protein	Flour protein	Ash	GP[1]	Break flour	Midds flour	Total flour[2]	Shorts	Bran	Total feed	Total production	Wt/bu[3]	Yield
%	%	%		%	%	%	%	%	%	%	*lbs*	*bu/bbl*
2° WHEAT												
14.2	13.5	0.45	420	17.75	53.40	72.20	6.65	20.90	27.55	98.70	63½	4.31.8
	13.5	0.46	404	17.95	53.75	72.55	7.00	20.10	27.10	98.80	—	4.30.0
	13.7	0.46	428	17.90	52.90	71.80	6.90	20.90	27.80	98.60	—	4.33.0
	13.5	0.45	420	17.70	52.90	71.50	7.30	20.80	28.10	98.70	—	4.34.0
	13.6	0.46	440	17.45	53.55	71.55	7.70	20.00	29.20	99.20	—	4.33.12
Av.	13.55	0.456	—	17.75	53.50	71.92	7.11	20.54	27.95	98.80	—	4.32.8
Standard deviation				0.20	0.39	0.45	0.40	0.45	0.78	0.23	—	—
3° WHEAT												
12.6	11.8	0.45	580	18.15	50.25	69.30	8.50	21.75	30.25	98.65	63	4.43.0
	11.9	0.45	596	18.00	50.70	68.50	9.65	22.10	31.75	100.45	—	4.46.0
	11.9	0.45	576	17.80	49.40	69.60	7.40	22.00	29.40	96.60	—	4.42.0
	11.7	0.45	576	17.85	50.30	68.90	9.00	21.90	30.90	99.05	—	4.44.8
	11.8	0.44	600	17.90	51.85	69.75	9.15	21.15	30.30	100.05	—	4.41.0
Av.	11.8	0.448	—	17.94	50.50	69.21	8.74	21.78	30.52	98.96	—	4.43.0
Standard deviation				0.16	0.91	0.51	0.85	0.38	0.87	1.50	—	—
4° WHEAT												
13.6	12.8	0.49	540	16.65	50.65	67.60	10.60	21.65	32.25	99.55	61	4.50.0
	13.0	0.49	552	16.40	50.15	67.10	10.90	21.90	32.80	99.35	—	4.52.0
	12.8	0.48	528	16.55	50.20	67.30	10.10	22.25	32.35	99.10	—	4.51.0
	12.7	0.48	526	16.35	51.65	68.90	9.65	21.15	30.80	98.80	—	4.44.8
	12.8	0.48	540	16.30	51.80	68.40	10.00	21.50	31.50	99.60	—	4.46.8
Av.	12.8	0.484	—	16.45	50.89	67.86	10.25	21.69	31.94	99.18	—	4.48.8
Standard deviation				0.15	0.79	0.76	0.42	0.41	0.79	0.33	—	

ANALYSIS OF VARIANCE

	Sum of squares	Degrees of freedom	Variance	*F*
Between grades	42.75	2	21.374	61.2
Error	4.187	12	.349	—
Total	46.937	14	—	—

[1] GP = gassing power (Sandstedt and Blish, Cereal Chem. 11: 381–2, 6 hours, 30°C).
[2] Total flour—computed on basis of total products.
[3] Wt/bu = weight per bushel—Imperial measure.

Duplication of Results

The factors which might cause variability probably exist within the mill itself and/or are a result of the technique of the operator. To determine the possibility of maintaining uniform milling conditions

TABLE VI

RANDOM MILLING RESULTS SHOWING EFFECT OF PHYSICAL CONDITION OF WHEATS WITHIN GRADES

Wt/bu[1]	Wheat protein	Flour protein	Ash	GP[2]	Total[3] flour	Total feed	Yield
lbs	%	%	%		%	%	*bu/bbl*
2° WHEATS							
62½	14.2	13.7	0.48	438	72.20	27.80	4.31.8
63	14.3	13.4	0.45	446	72.50	27.75	4.30.8
63	14.3	13.5	0.46	450	72.70	27.55	4.29.8
63	14.3	13.7	0.45	380	72.80	28.70	4.29.0
62½	14.2	13.6	0.47	380	72.85	27.25	4.29.0
62½	14.1	13.3	0.46	436	72.15	27.90	4.31.8
62	13.9	13.2	0.46	342	72.00	28.38	4.32.0
63	13.7	13.2	0.46	354	71.75	28.15	4.33.0
62	14.2	13.7	0.47	444	73.10	26.60	4.28.0
62½	14.2	13.4	0.47	400	72.30	27.50	4.31.0
Av.	14.1	13.5	0.463	—	72.43	27.76	—
Standard deviation			—	—	0.41	0.59	—
3° WHEATS							
62	13.9	13.4	0.47	360	70.60	29.35	4.37.8
61	14.1	13.7	0.47	438	69.80	30.00	4.41.0
62	13.8	13.3	0.45	424	71.60	28.40	4.33.8
62	13.1	12.5	0.45	508	71.00	29.40	4.36.0
62	12.8	12.0	0.46	530	69.70	30.10	4.41.0
61	14.1	13.3	0.45	390	70.10	29.80	4.39.8
61	13.2	12.5	0.46	364	70.40	29.50	4.38.0
61	13.1	12.2	0.47	520	70.25	29.75	4.39.0
61	15.7	14.9	0.46	500	72.10	28.00	4.32.0
62	14.3	13.8	0.47	340	71.75	27.75	4.32.8
Av.	13.8	13.15	0.46	—	70.73	29.20	—
Standard deviation			—	—	0.84	0.85	—
4° WHEATS							
61½	13.8	12.7	0.49	518	69.30	30.50	4.43.0
60	13.9	12.9	0.49	576	67.25	32.30	4.51.8
60½	14.2	13.5	0.49	416	69.70	30.25	4.41.0
60	14.3	13.8	0.49	438	70.10	29.85	4.40.0
60	13.7	12.9	0.49	490	68.70	31.40	4.45.0
60½	13.3	12.6	0.48	610	67.00	33.30	4.52.8
60½	13.6	12.7	0.50	440	69.10	31.35	4.43.8
60	13.7	12.8	0.48	410	67.39	33.05	4.50.12
60	13.5	12.5	0.48	528	67.10	33.25	4.52.0
60	13.6	12.7	0.48	464	69.50	30.15	4.42.0
Av.	13.75	12.9	0.487	—	68.51	31.54	—
Standard deviation		—	—	—	1.20	1.35	—

[1] Wt/bu = weight per bushel—Imperial measure.
[2] GP = gassing power (Sandstedt and Blish, Cereal Chem. 11: 381–2, 6 hours, 30°C).
[3] Total flour = computed on basis of total products.

and securing duplicate results, three samples of replicate millings were made. The first, second, and third samples contained five replicate millings each of 2°, 3°, and 4° wheats respectively. The flour yields of these millings were reduced by means of statistical analysis. The method of analysis of variance is described by Goulden.[2]

The error within the samples was found to be not significant and indicates that milling results can be duplicated on the Buhler. The variation between the grades is large and shows the effect of the physical character of the wheat upon the flour yield.

The accuracy of mill control which bears some relation to the skill and care of the operator is indicated in the standard deviation of the shorts and bran. It will be noted that the error of replication is not necessarily higher among the lower grades of wheat. Duplication of milling results can therefore be obtained with sufficient accuracy in any grade of wheat.

TABLE VII

SUMMARY OF MILLING RESULTS OF VARIOUS STATUTORY GRADES

Grade	Wt/bu	Yield	Yield	Ash	Break flour	Feed	No. samples
		%	*bu/bbl*	%	%	%	
1°	63½	73.48	4.27.0	0.452	16.86	26.75	7
2°	62½	73.15	4.28.0	0.464	17.30	26.70	50
3°	62	71.59	4.33.12	0.465	17.00	28.30	19
4°	61	69.08	4.43.12	0.480	15.70	30.80	18
Garnet	64½	71.46	4.34.8	0.493	13.75	28.00	16
Special	56	68.89	4.47.0	0.510	16.70	30.90	10

In Table VI, are shown milling results of 2°, 3°, and 4° wheats respectively. These results were taken at random from among numerous millings of wheat shipments during a single crop year. The variation within the samples increased with each decrease in grade. The tendency of the physical character of the lower grades of wheat to vary more widely indicates that wheats of similar statutory grade may be classified further according to their respective milling values. As far as the statutory grades are concerned, it is usually the case that wheats of poorest milling value possess the poorest baking quality.

Table VII shows a summary of the milling results of various grades of wheats. They indicate that the flour yield is influenced by the physical condition of the wheat rather than by the weight per bushel.

Correlation of the Buhler and Commercial Milling Results

The analysis of the Buhler milling results in Table V indicated that the error of replication was not significant. A survey of a series of commercial milling results indicated that the error of replication

[2] C. H. Goulden: Methods of statistical analysis, John Wiley and Sons, New York, 1939.

was smaller than that of the experimental mill. It was concluded, therefore, that a correlation with respect to flour yield should exist between the Buhler and commercial units. As a preliminary experiment, it was arranged to mill a sample blend of wheat on the commercial unit for six consecutive days. Samples of the wheat blend and straight-grade flour were taken daily and the flour yields (total products) for each day were calculated. The six samples of the wheat

TABLE VIII

COMPARATIVE ANALYSES OF COMMERCIAL AND BUHLER FLOURS

Wheat protein	Flour protein	Ash	GP[2]	Flour[1]	Feed	Total products	Yield[3] as found	Moisture as rec'd	Yield
%	%	%		%	%	%	%	%	*bu/bbl*
				BUHLER					
14.2	13.3	0.46	460	73.6	26.00	98.50	76.23	10.4	4.26.5
14.1	13.3	0.46	460	73.4	26.05	98.25	75.85	10.6	4.27.0
14.2	13.4	0.48	480	72.0	27.60	98.70	74.16	10.9	4.33.9
14.2	13.3	0.48	450	72.0	27.40	98.20	74.41	10.6	4.33.9
14.2	13.3	0.47	492	73.0	26.55	98.30	75.44	10.6	4.28.8
14.2	13.4	0.48	510	72.0	27.50	98.20	74.50	10.5	4.33.9
Av. 14.2	13.3	0.47	475	72.67	26.85	98.36	75.10	10.6	4.29.11
				COMMERCIAL					
14.2	13.5	0.46	500	73.2	26.8	100.0	74.40	12.4	4.27.11
14.2	13.5	0.45	500	73.6	26.4	100.0	74.49	12.4	4.26.5
14.2	13.5	0.46	460	73.3	26.7	100.0	74.20	12.5	4.27.5
14.2	13.6	0.46	496	73.2	26.8	100.0	74.50	12.0	4.27.11
14.2	13.6	0.46	520	73.15	26.85	100.0	74.12	12.4	4.27.15
14.2	13.7	0.46	520	72.85	27.15	100.0	73.75	12.6	4.29.0
Av. 14.2	13.55	0.46	499	73.21	26.79	100.0	74.18	12.4	4.27.11

[1] Total flour computed on basis of total products.
[2] GP—gassing power (Sandstedt and Blish, Cereal Chem. 11: 381–382, 6 hours, 30°C).
[3] Yield—"as found" moisture basis.

blend were then conditioned according to experimental method and milled on the Buhler. The data covering the six days' operation of the commercial unit and the corresponding results of the Buhler millings are shown in Table VIII. The yield results were reduced by statistical analysis. The theory involved in the determination of the correlation coefficient and the test of significance of the coefficient is described by Goulden.

$$\Sigma(x^2) = 45.52 \qquad \Sigma(y^2) = 62.375$$
$$T^2_x/6 = 42.67 \qquad T^2_y/6 = 62.0817$$
$$\Sigma(x-x)^2 = 2.85 \qquad \Sigma(y-y)^2 = .2933$$
$$\Sigma(xy) = 52.18$$
$$T_xT_y/6 = 51.467$$
$$\Sigma(x-x)(y-y) = .713$$

$$r_{xy} = \frac{.713}{2.85 \times .2933} = .854$$

To determine the significance of r_{xy}

$$t = \frac{r\sqrt{n}}{\sqrt{1 - r^2}} = 3.28 \qquad n = 4 \text{ (degrees of freedom)}$$

x = Buhler yield $\qquad$ y = commercial yield

Since t at the 5% level of probability equals 2.78, the correlation is statistically significant.

The covariation of the Buhler and commercial flour yields is shown in the regression graph, Figure 2. The regression equation is $Y = 54.54 + .257x$, where Y equals the estimated values of y.

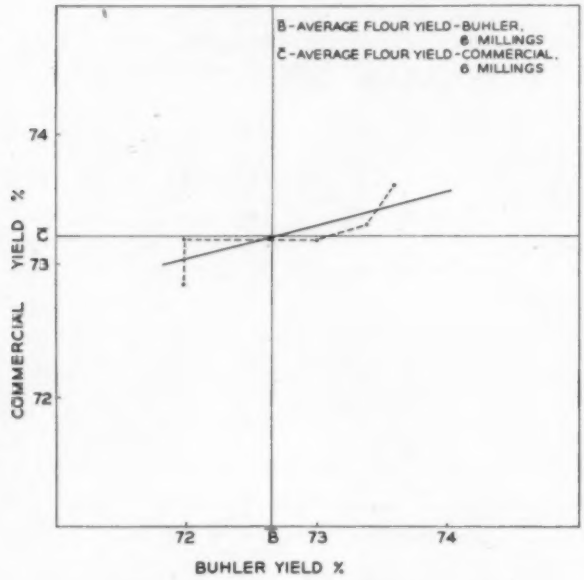

Fig. 2. Regression graph of comparative flour yields.

The above series of milling tests was repeated and although there was a slight increase in error of replication in the Buhler results, the correlation was still significant.

It appears probable that experimental error can be minimized sufficiently by the proper technique and care of the operator so that a significant correlation would exist.

A Comparison of the Buhler and Commercial Flours

The respective straight-grade flours were accepted as a basis for comparison, since it was found that certain characteristics are apparent in the break flour and are peculiar to different grades of wheat.

The standard A. A. C. C. baking formula was used but with 200 g of flour (13.5% moisture basis), also a bromate (0.001%) and fermentation differential (2 hours, 40 minutes: 3 hours, 40 minutes). In the initial test, four loaves were baked from each flour. A modification included the addition of a malt supplement depending on the gassing power of the experimental flour. Other supplementary bakings included modifications of the fermentation time, depending on the protein content of the flour. The flours milled during the six days' operation of the commercial unit and the corresponding flours milled on the Buhler were analyzed and baked. The protein conversion

TABLE IX

COMPARATIVE LOAF VOLUMES OF BUHLER AND COMMERCIAL FLOURS

Fermentation time	Buhler volume		Commercial volume	
	Initial[1]	Stimulated[2]	Initial[1]	Stimulated[2]
2 hours, 40 minutes	cc	cc	cc	cc
	690	720	700	725
	695	715	710	730
	710	715	695	720
	680	725	715	725
	690	710	705	720
	710	730	710	720
Average	696	720	706	723
3 hours, 40 minutes	660	685	685	685
	630	680	670	665
	630	700	665	670
	645	685	675	685
	650	700	675	685
	650	680	660	680
Average	644	688	671	678

[1] Initial: Standard A. A. C. C. formula.
[2] Stimulated: A. A. C. C. formula plus 10 ppm $KBrO_3$ (0.001%).

(difference between wheat and flour protein) of the two flours was similar and the ash contents compared favorably. Approximately the same flour extraction, therefore, was obtained in each case.

The flours were stored in a fermentation cabinet until they attained a moisture content of 13.5%. The absorption was then determined by mixing a few trial doughs. The same absorption was used for each flour and a dough of similar consistency was obtained in each case. Dough handling properties seemed much the same throughout the samples. The loaf volumes are given in Table IX. A summary of the baking analyses indicated a definite similarity with respect to loaf volume, crust, and crumb character.

Flour Color

Although we have not determined the respective carotene contents, the color of the Buhler flour appears somewhat more yellow than that of the commercial, but color comparisons among experimental flours are readily made.

Granulation

The Buhler flour is more granular to the feel than the commercial flour even though the actual granulation is similar, but this condition does not appear to cause any wide discrepancies in either the baking test or the gassing power. The gassing power of the commercial flour may be reliably predicted on the basis of experimental results.

Summary of Milling and Baking Value

Our results of an experimental milling and baking test are summarized as to milling and baking value. The milling value includes the classification of the wheat with respect to the physical condition of the kernels, the observations recorded of the milling behavior, and the flour yield. The observations of the milling behavior of the grain are of course limited, due to the short system and the lack of classification of stocks. The baking value is primarily an interpretation of the "blending value" and "carrying capacity" of the wheat with respect to its value in a commercial blend.

Milling Technique

A number of points are considered important with respect to milling technique and may be enumerated as follows:

1. A preliminary warm-up of the mill is considered necessary since adjustments of roll settings were made when the mill was warm.

2. The feed gate above second reduction roll should be adjusted to back up the stock sufficiently to maintain an even and steady rate of feed to the roll. This has much to do with having the proper load on the tail of the mill.

3. Since the mill does not carry a full load at the start of a run, the abrasive action on the stocks tends to throw the flour off color. At the end of the run the same action occurs, and in addition when the mill is swept out, any accumulation of foreign material is swept into the flour, with the result that the flour is dark in color and high in ash. Portions of flour are therefore taken off at the first and last of a run, weighed up and added into the gross weight in computing the yield. The flour produced during the middle of the run is used for color, chemical, and baking tests.

4. It was noticed that regardless of how well the mill was swept out, after a number of millings there was an accumulation of foreign material within the mill which appeared to get into the flour occasionally and affect the color. It is considered wise therefore to thoroughly "blow out" the mill with compressed air after each day's milling.

Relative Humidity

Humidity conditions in our mill room vary between 50% and 90% in winter and summer. These extreme conditions appear to have an effect on our milling results. Higher humidity tends to decrease the capacity and bolting efficiency of the mill. Although we have been unable to experiment in this connection, it would appear that milling conditions in an air-conditioned mill room would be much better controlled.

Summary

Although the personal factor is evident, the duplication of milling results with the Buhler is possible under controlled conditions and technique. Little adjustment of the primary mill set-up is required when grinding wheats of varying weight per bushel, apart from regulation of rate of feed.

The error of replication in both the experimental and commercial millings was found not to be significant. The flour characteristics and baking values of the Buhler and commercial flours were sufficiently comparable for reliable interpretation. It is concluded then that the Buhler mill is useful for predetermining commercial milling and baking results.

A CRYSTALLINE PROTEIN OBTAINED FROM A LIPOPROTEIN OF WHEAT FLOUR

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The occurrence of a curious, hitherto unsuspected sulphydryl compound in wheat flour was reported recently from this laboratory by Balls and Hale (1940). The substance, apparently in combination with a lecithin-like lipoid, was extracted from flour by petroleum ether. The extract, after some purification, was hydrolyzed with hydrochloric acid and then yielded a sulfur-containing material which no longer resembled a lipoid, but behaved like a protein. This material was

[1] Enzyme Research Laboratory Contribution No. 71.

soluble in water and dilute alcohol, but not in fat solvents; from aqueous solutions it could be salted out by ammonium sulfate; it contained roughly 13% of nitrogen and no measurable amount of phosphorus. Nearly 3% of the material was sulfur, no longer in the $-SH$ form, but present as cystine.

Further investigation has shown that such material is not a single substance. Considerable variation in the content of nitrogen and sulfur has been encountered between different preparations. One of the substances present was, however, found to remain in solution in 70% ethyl alcohol, but to precipitate when the concentration of alcohol was increased to about 90%. On repetition of this treatment, the precipitate was crystalline. The crystals formed rosettes of needles, somewhat reminiscent of tyrosine. Two subsequent recrystallizations did not change the apparent crystal form or cause a significant change in the content of sulfur or nitrogen. One constituent of the original materials is thus obtainable in reproducible form. The purpose of this paper is to report observations made in a chemical study of this crystalline substance.

Method of Preparation

The following example will serve to illustrate the method of preparation:

200 pounds of freshly milled unbleached patent flour from soft wheat was extracted with high-grade petroleum ether in a percolator. Nearly all the solvent was removed from the extract by distillation in vacuum; the liquid extract was kept at $-1.5°$ C for several weeks and then separated in a refrigerated centrifuge from crystals of sterol that had deposited during cold storage. The liquid was next mixed with an equal volume of ether and then with three volumes of cold 1*N* hydrochloric acid in absolute ethyl alcohol. The mixture stood at 0° for an hour; then the precipitate that formed was separated in a centrifuge and thoroughly washed, first with absolute alcohol and then with ether. After drying, 25.2 g of material comparable to that described in our previous paper was obtained.

This crude material, dissolved in 100 ml of water, was mixed with 300 ml of absolute alcohol. The precipitate that formed was removed in the centrifuge, and the supernatant liquid was evaporated somewhat on the steam bath and finally to dryness in vacuum over P_2O_5. The dry residue weighed 16.8 g.

15.5 g of this residue was dissolved in 25 ml of water; 225 ml of absolute alcohol was then added and the mixture was allowed to stand at 5° for 4 hours. An entirely crystalline precipitate formed, whose weight after drying was 4.10 g. The crystals are shown in Figure 1.

Fig. 1. Protein obtained from a lipoprotein of wheat flour.

Recrystallization was made in the same manner as the step just described. For the analytical work reported here thrice crystallized material was used. Its composition was essentially the same as that of material twice crystallized, as shown by the data in Table I.

TABLE I

COMPOSITION AFTER RECRYSTALLIZATION

	Nitrogen	Chlorine	Sulfur
	%	%	%
Crystallized once	16.20	6.64	4.49
			4.31
Crystallized twice	17.25	6.56	4.46
	17.50	6.57	
Crystallized thrice	17.45	—	—
	17.10		

Analytical Results

The crystalline material was found to be very soluble in water, and in dilute alcohol. The water solution gave a positive biuret and a

positive Molisch test.[2] Hydrolysis in constant-boiling HCl (120°) for six hours produced nearly an eightfold increase in amino nitrogen.[3] The amino nitrogen after hydrolysis was also about three-fourths of the total nitrogen originally present. The data appear in Table II. Hydrolysis for 18 hours instead of 6 caused no significant change in the results.

TABLE II

AMINO NITROGEN BY VAN SLYKE METHOD

	Sample	Amino nitrogen
	mg	%
Original material	4.75	1.65
	4.75	1.65
		Av. 1.65
After acid hydrolysis[1]	2.63	11.99
	2.63	13.03
	5.25	12.50
	5.25	12.60
		Av. 12.53

[1] Boiled 6 hours with 20% HCl at 120°; samples from a stock solution of the hydrolysate, represented 2.63 mg original substance per milliliter.

A very small droplet of heavy, oily liquid always remained after hydrolysis. The amount of this "oil" was not great enough to affect the analytical results appreciably. The "oil" did not appear to diminish when the period of hydrolysis was extended from 6 to 18 hours, and the droplet was insoluble in strong, hot solutions of either HCl or NaOH.

Determinations of individual constituents were made by the methods and with the results shown in Table III. On the basis of these determinations the equivalent proportions were computed, and are shown in Table IV. Ammonia was absent from the hydrolysate, indicating the absence of acid amide nitrogen in the original.

Probable Molecular Weight

A minimum value for the molecular weight of the substance may be calculated from the data of Table IV. Assuming the presence of one tyrosine residue in the molecule, this minimum is approximately 6,000.

With such molecules, it is customary to attempt an estimation of the size by determination of the rate of diffusion. To this end the technique of Northrop and Anson (1928–29) was used. The diffusion cell was calibrated with 2*M* NaCl.

[2] A test with the orcin reagent of M. Sörensen indicated that the amount of carbohydrate present was less than 0.5%.

[3] Determined in the volumetric apparatus of Van Slyke after 3 minutes' shaking with the customary nitrous acid reagent. Shaking for 10 minutes gave the same result.

A solution of 3.33 mg per ml in 0.5*M* NaCl was allowed to diffuse for 26 hours against an equal volume of 0.5*M* NaCl. Nitrogen determinations gave a diffusion coefficient of 0.155 cm²/day, a molecular radius of 1.44×10^{-7} cm, and thus a molecular weight (assumed density = 1.3) of 10,200. This value is an indication that under the conditions described, the molecular weight is about 12,000, namely twice that calculated from the analyses.

TABLE III

ANALYSIS OF THRICE-CRYSTALLIZED MATERIAL

Component sought	Method used	Sample taken	Amount found
		mg	%
Chlorine	Pregl (1937)	22.860	6.57
		20.215	6.56
Sulfur	Pregl (1937a)	23.900	4.49
		23.500	4.31
		21.789	4.46
Nitrogen	Kjeldahl after Pregl (1937b)	4.409	17.22
		3.279	17.32
		2.797	17.45
Phosphorus	Fiske and Subbarow (1925)	2.00	None[1]
Selenium	A. O. A. C. (1940)	50.0	None[2]
Ash	Residue from a chlorine determination	11.036	0.06
Arginine	Thomas, Ingalls and Luck (1939)	12.68	20.4
Cystine	Sullivan and Hess (1930)	11.43	15.7
Tyrosine	Bernhart (1938)	16.79	3.0
Tryptophane	Block (1938)	16.79	Trace[3]

[1] Therefore less than 0.05% phosphorus.

[2] The method is said to detect 3×10^{-7} mg of selenium. The authors wish to thank Mr. Hubert W. Lakin of the Bureau of Plant Industry for making the test.

[3] The tryptophane determination was carried out on the $HgSO_4$ precipitate from the tryosine determination. The color value corresponded to 0.3% tryptophane, but was probably due to a trace of tyrosine. The value obtained for the molecular weight makes it probable that the finding of any amino acid in so small an amount is an error.

TABLE IV

CONSTITUTION OF THRICE-CRYSTALLIZED SUBSTANCE

Constituent	Percent of weight	Percent of total N	Proportion of equivalents[1]
Chlorine	6.57	—	11.0
Sulfur	4.42	—	8.2
Nitrogen	17.35	(100)	73.8
Amino nitrogen by Van Slyke method	1.65	9.5	7.0
Arginine	20.4	37.8	6.9
Cystine	15.7[2]	10.5	3.9
Tyrosine	3.0	1.3	1.00
Unaccounted for[3]	—	40	—

[1] Based on tyrosine = 1.00.

[2] Equivalent to 4.18% sulfur, or 95% of the total sulfur found. It is thus probable, though not proved, that all the sulfur exists as cystine.

[3] The assumption is made that the Van Slyke amino nitrogen is not derived from arginine. If the difference between original total nitrogen and amino nitrogen after hydrolysis (see Table II) is regarded as due entirely to proline, 28% of the total nitrogen would be proline, leaving only 12% unaccounted for. The several serious errors probably inherent in such speculation all tend to make the value for the supposed proline too high; nevertheless, proline or a related group may well be present in a very considerable amount.

A molecule of this size could scarcely be expected to give a measurable sedimentation constant in our ultracentrifuge. In an experiment in which a solution of crystals in water containing 3.1 mg per ml was centrifuged for 3 hours at a mean centrifugal force of 91,000 × gravity, no sedimentation was in fact observed.

Behavior in the ultracentrifuge thus appears to agree with the diffusion measurement, but it must be admitted that both methods require unwarranted assumptions with regard to the shape of the particles. The computations have here been based on spherical particles.

Remarks on Constitution

It may be argued with fair success that the only completely inclusive description of a protein is a substance that yields amino acids on hydrolysis. The production of amino acids by hydrolysis of this crystalline material from wheat shows that a good part of the molecule has the composition ascribed to proteins or peptides. Well over half the total nitrogen has been definitely accounted for as arginine, cystine, and tyrosine. Some of the remainder is probably proline or a related substance; but the existence of a nitrogen-containing group which is not an amino acid has not been excluded.

A formal distinction between protein and nonprotein nitrogen is often based on behavior toward trichloracetic acid. The usual method of separating protein from nonprotein nitrogen is to precipitate the former from solution by the addition of trichloracetic acid to a concentration of 0.15*M*. The mixture is then held briefly at 70°, and the precipitated protein is finally separated. Under such conditions a half-percent solution of the crystals in question gave no precipitate, but when the concentration of trichloracetic acid was increased to 0.4*M*, 97% of the nitrogen was precipitated. The precipitate, however, completely redissolved on warming, and reappeared on cooling.

It is evident that the substance lies on the borderline between true proteins and bodies of smaller molecular dimensions. One point of resemblance to proteins is the ease with which solutions undergo some change akin to polymerization or denaturation, whereby the substance becomes no longer readily soluble in water or dilute acid. Such a change occurs when a solution in water is electrolyzed. The substance naturally gives positive results with the biuret, xantho-proteic, and Millon tests. It will probably be more convenient to refer to the substance as a protein, if the word is not permitted to imply too much.[3]

The crystals are evidently a hydrochloride. Silver chloride may be precipitated directly by adding silver nitrate to an aqueous solution.

[3] The authors will appreciate any carefully made suggestion of a name for this substance.

The acidity of such aqueous solutions is furthermore very high. A solution of 1 mg per ml in water was found to be at pH 3.85. This value approximates a 5% dissociation of all the chlorine (if no other acid groups interfered). The base is therefore a strong one, in spite of its high cystine content.

Substances resembling our material from wheat in molecular size and general make-up are well known, but examples are not very common. Among such are the protamines, the inhibitors of trypsin and of pepsin, and the bactericidal substances recently described by Dubos and Cattaneo (1939).

The protamines, like our material, are strongly basic and contain much arginine. We are not aware, however, of any recognized protamine that contains cystine. Trypsin inhibitor, crystallized and studied by Kunitz and Northrop (1936), has the general properties of a polypeptide with a molecular weight of about 6,000. The inhibitor of pepsin, crystallized and studied by Herriott (1941), also contains amino acids and resembles our material in size (mol wt 4,000–10,000) and in high content of arginine. Two crystalline substances, gramicidin and tyrocidin, isolated from a soil microörganism by Dubos and Cattaneo (1939) and Hotchkiss and Dubos (1940), are composed largely of amino acids. They appear to be smaller molecules than the substance with which we have worked. Gramicidin is soluble in acetone and in a mixture of acetone and ether, and has a molecular weight of 1400. Tyrocidin is soluble in hot absolute alcohol. Sulfur has not been reported as a constituent of either body, but tyrocidin forms a crystalline hydrochloride. There is a point of resemblance between these substances and that isolated from wheat in that the latter is also a very powerful bactericide. This property will be reported in detail in a later paper.

The material isolated from wheat is thus quite distinct from the somewhat similar substances just discussed. In the grain, it undoubtedly exists in a reduced form containing $-SH$ groups. There can be little question that such an $-SH$ compound in wheat and flour increases the activity of the papain-like enzyme present in both. The substance is apparently a natural activator of the wheat proteinase.

The original solubility of the substance in petroleum ether shows that it occurs in combination with a lipoid. Such solubility is not a mixed-solvent effect. The mere presence of flour lipoids in solution in gasoline or ether was not sufficient to redissolve the protein-like body, once that had been prepared in the water-soluble state. Nor is the solubility in petroleum ether due to the original reduced condition of the substance, for it remains soluble after the $-SH$ groups have been oxidized by air. Thereafter it may be precipitated, along with some

lipoidal material, by ethyl acetate or by alcohol. The precipitate made with acetate is easily soluble again in petroleum ether or in ether, and may be precipitated and redissolved repeatedly.

The ethyl acetate precipitate was found to contain about 1% of phosphorus. It was only partly soluble in hot absolute alcohol and the insoluble fraction had roughly the same phosphorus content as the original. Continued treatment with hot neutral alcohol slowly removed the phosphorus from the insoluble fraction, but the addition of hydrochloric acid to the alcohol caused rapid removal, even in the cold. An experiment is detailed in Table V. It should be remembered that this ethyl acetate precipitate contains other protein-like substances, apparently similar to, but not identical with, the one that was crystallized as is reported here.

TABLE V

BEHAVIOR OF AN ETHYL ACETATE PRECIPITATE

Treatment	Wt of fraction	Composition N	Composition P
	mg	%	%
(1) Original[1]	1,000	4.21	1.15
(2) One gram of (1) heated to 78° in 25 ml of absolute alcohol, centrifuged at 60°–70°. Sediment only	110	8.58	0.99
(3) Sediment (2) resuspended 25 ml of boiling alcohol for 15 min and recentrifuged. Sediment only	—	10.4	—
(4) Sediment (3) boiled 30 min in 25 ml of absolute alcohol, centrifuged. Sediment only	—	10.4	0.57
(5) Part of sediment (4) suspended in absolute HCl alcohol (1*N*) at 50° for 15 min. Centrifuged, sediment only	—	11.7	0.16
(6) 95 mg of sediment (4) suspended in 2 ml 1*N* HCl alcohol at 0° and kept at 0° for 2 days. Sediment washed well with dry ether	59	10.8	0.28

[1] Precipitated by adding 3 volumes of ethyl acetate to the liquid portion of the flour lipoids as originally extracted by petroleum ether. Precipitate washed 3 times (centrifuge) with 1 volume of cold ethyl acetate.

It is unlikely that a free lipoid like lecithin can act in this manner toward ethyl acetate, hot alcohol, and cold acid-alcohol. In any case, repeated treatments with large volumes of boiling alcohol should remove lecithin. A lecithin-like lipoid in combination with a protein, however, might behave in the manner observed.

In view of the basic nature of our substance, a reasonable working hypothesis seems to be that it is either associated with or replaces choline or other base in a lecithin-like lipoid. Whatever the form of connection between protein-like fraction and lipoid may be, it seems evident that the mother substance of our crystalline product belongs

in the vaguely defined class of lipoproteins. It is thought that a beginning has been made in the description of one fragment of a lipoprotein.

Summary

A crystalline substance of protein-like nature has been obtained from the petroleum ether extract of wheat flour. Analytical examination has shown that the substance consists mainly of amino acid residues. Arginine, cystine, and tyrosine account for over half of the total nitrogen. The crystals are a hydrochloride of a basic substance. This hydrochloride has a minimum molecular weight of 6,000, and a probable molecular weight of double that value. The substance therefore lies in the borderline between proteins and polypeptides, where such protein tests as precipitation with trichloracetic acid are indefinite. In the grain and in flour the material exists in the reduced form as a sulphydryl compound. It is probably a natural activator of the wheat proteinase. The method of isolation and the behavior of the substance during purification lead to the belief that the protein-like substance exists in flour combined with a phosphorus-bearing lipoid, and is therefore a component of one member of the little understood class of lipoproteins.

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BACTERICIDAL AND FUNGICIDAL PROPERTIES OF A CRYSTALLINE PROTEIN ISOLATED FROM UNBLEACHED WHEAT FLOUR

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The discovery of "in vitro" and "in vivo" bactericidal activity for certain crystalline polypeptides by Dubos (1940), Dubos and Hotchkiss (1941), and Dubos and Cattaneo (1939) suggested the study of a somewhat similar crystalline protein recently separated from the petroleum ether extract of unbleached wheat flour for similar properties. Balls, Hale, and Harris (1942) separated and gave a rather complete chemical description of this protein. It differs from the alcohol-soluble, water-insoluble polypeptides designated as "gramicidin" and "tyrocidin" by Hotchkiss and Dubos (1940) and Dubos and Hotchkiss (1941) in that it is water-soluble, alcohol-insoluble, contains about 4.5% sulfur in the form of cystine, and is completely precipitated by 2.5% trichloracetic acid.

It appears to be the oxidized form of a powerful oxidation-reduction system and, therefore, might be expected to have considerable biological activity.

"In Vitro" Bactericidal Tests

In the "in vitro" testing for bactericidal activity the selection of a suitable method is always a "moot" question. Results by any single method may fail to give a complete picture of the limits of activity. For this reason three divergent methods were employed in these studies. These methods may be designated as: (1) the broth culture

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TABLE I

EFFECT OF CRYSTALLINE PROTEIN ON BACTERIA IN BEEF-HEART INFUSION AND GLUCOSE BROTH AFTER INCUBATION AT 37.5°C FOR 48 HOURS

Protein added as the hydrochloride	Test Organism: *Streptococcus viridans*		*Pneumococcus Type I*		*Pneumococcus Type III*		*Sarcina lutea*	
	Bacteriostasis	Viability	Bacteriostasis	Viability	Bacteriostasis	Viability	Bacteriostasis	Viability
mg per ml								
0.5	−	+	−	+	−	+	−	−
0.25	−	+	−	+	−	+	−	−
0.10	−	+	−	+	−	+	−	−
0.05	−	+	−	+	−	+	−	−
0.025	−	+	−	+	−	+	−	+
0.001	−	+	+	+	+	+	−	+
0.0005	+	+	+	+	+	+	+	+
0.00	+	+	+	+	+	+	+	+

Protein added as the hydrochloride	Test Organism: *Staphylococcus aureus*		*Escherichia coli*		*Eberthella typhi*		*Pseudomonas pyocyaneus*	
	Bacteriostasis	Viability	Bacteriostasis	Viability	Bacteriostasis	Viability	Bacteriostasis	Viability
mg per ml								
0.5	−	+	+	+	+	+	+	+
0.25	−	+	+	+	+	+	+	+
0.10	−	+	+	+	+	+	+	+
0.05	−	+	+	+	+	+	+	+
0.025	+	+	+	+	+	+	+	+
0.001	+	+	+	+	+	+	+	+
0.0005	+	+	+	+	+	+	+	+
0.00	+	+	+	+	+	+	+	+

+ = growth.
− = no growth.

method of Dubos and Cattaneo (1939); (2) the agar-cup plate method of Reddish (1929); and (3) the accelerated death curve method of Cade and Halvorson (1934).

With the broth culture method *Streptococcus viridans*, *Pneumococcus Type I*, *Pneumococcus Type III*, *Staphylococcus aureus*, *Sarcina lutea*, *Eberthella typhi*, *Escherichia coli*, and *Pseudomonas pyocyaneus* were employed as test organisms. The crystalline protein hydrochloride was added to tubes of nutrient beef-heart infusion and glucose broth [3] after sterilization. The amounts added were equivalent to 0.5, 0.25, 0.1, 0.05, 0.025, 0.001, and 0.0005 mg per ml of broth. Control tubes of broth without added protein hydrochloride and tubes con-

[3] 1,000 ml beef-heart extract and 1,000 ml distilled water containing 10 g NaCl, 0.4 g $MgSO_4$, 2.0 g K_2HPO_4, 0.2 g $CaCl_2$, 20 g bactopeptone, and 2.0 g of glucose; adjusted with NaOH to pH 7.5; sterilized at 15 lbs for 20 minutes.

taining the amounts specified above were each inoculated with one loop of inoculum and incubated at 37.5°C for 48 hours. The loop inocula were taken from 48-hour cultures of the test organisms in Brewer's (1940) semisolid thioglycolate broth.

After 24 and 48 hours of incubation all broth cultures were examined for visible signs of growth. The absence of visual signs of growth was interpreted as evidence of bacteriostasis. After 48 hours all test cultures were subcultured, using a loop needle transfer back into thioglycolate broth. These subcultures were in turn incubated for 48 hours at 37.5°C. Absence of growth in subcultures was interpreted as evidence of bactericidal activity. The results of tests using this method are recorded in Table I.

As shown in Table I only one of the test organisms was completely killed—namely, *Sarcina lutea*. However, all of the Gram-positive organisms were definitely inhibited with the higher concentrations of the protein. The Gram-negative organisms *Escherichia coli*, *Eberthella typhi*, and *Pseudomonas pyocyaneus* were neither inhibited nor killed. Thus, based on this method, the protein would appear to have slight bactericidal activity and strong bacteriostatic action only so far as Gram-positive organisms are concerned.

With the agar-cup plate method, the agar medium used was made up at pH 6.9 according to the latest directions given by Ruhle and Brewer (1931). Only two test organisms were employed—*Staphylococcus aureus* and *Eberthella typhi*. In each case six tubes each containing 15 ml of sterile melted agar, were cooled to 45°C, after which they were inoculated with 0.1 ml of a 24-hour plain broth culture. An individual plate was poured from each tube and the agar was allowed to solidify at room temperature. Disks were then reamed out of the solidified agar by means of a sterile metal cork borer, leaving cups. Into these cups 0.5-ml portions of the solutions to be tested were placed. Water solutions containing 1.0, 0.5, 0.25, 0.10 and 0.05 mg of the crystalline protein hydrochloride were used. All plates were incubated for 24

TABLE II

RESULTS OF AGAR-CUP PLATE TESTS FOR BACTERICIDAL ACTIVITY

Amount of protein hydrochloride in cup	Test organism	
	Staphylococcus aureus inhibition zone	*Eberthella typhi* inhibition zone
mg per ml		
0.500	present	present
0.250	"	"
0.120	"	"
0.050	"	"
0.025	"	absent
0.000	absent	"

hours at 37.5°C under unglazed porcelain tops to prevent the condensation of water of syneresis. The results of these tests are given in Table II. Figure 1 illustrates clearly the types of zones of inhibition obtained around the agar-cups with each test organism as indicated in Table II. From Table II it can be seen that zones were formed with the Gram-positive *Staphylococcus aureus* with all concentrations of the protein tested, whereas with the Gram-negative *Eberthella typhi* no zone was formed with 0.5 ml of solution containing 0.025 mg per ml of the protein. In no instance did inhibition appear to be complete.

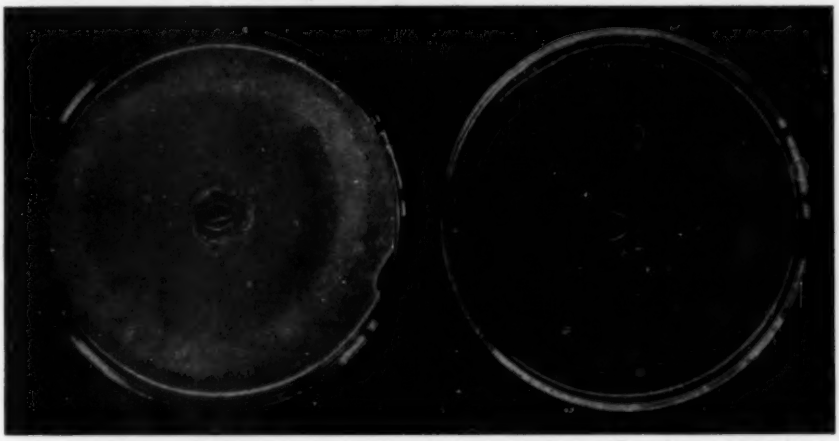

Fig. 1. Agar-cup plate tests for bactericidal activity of protein showing zone of inhibition around the cups with *Staphylococcus aureus* (A) and *Eberthella typhi* (T).

Careful examination with a hand lens showed either films of growth or small colonies within these zones. Another point of interest is that around the zone of inhibition there was, in the case of each organism, a narrow ring of very heavy growth that would indicate stimulation of cell multiplication by this protein at very low concentrations. The presence of growth films in the inhibited zones and the narrow rings of stimulated growth are shown in Figure 1.

Positive results by this agar-cup plate method are usually considered to be indicative of actual bactericidal activity. Thus, on the basis of the results obtained, true bactericidal action for both Gram-positive and Gram-negative bacteria can be claimed. Possibly the lower pH of the nutrient agar employed in these tests, as compared to that of the nutrient beef-heart infusion and glucose broth used in the previous tests, was responsible for the greater bactericidal activity shown.

Indications obtained by the agar-cup plate method of actual bactericidal activity were confirmed by results of tests using the accelerated death curve method of Cade and Halvorson (1934). In these studies the two test organisms *Staphylococcus aureus* and *Eberthella typhi* were again employed. The procedure was as follows:

Solutions to be tested were made up by dissolving the crystalline protein hydrochloride in sterile distilled water to give concentrations equivalent to 1.0, 0.5, 0.25, 0.10, 0.05, 0.01, 0.005 and 0.001 mg per ml. These solutions and control solutions of sterile distilled water were transferred to sterile, lipped 25 × 150-mm "Pyrex" test tubes. These tubes were then suspended in a constant-temperature water bath at 30°C and allowed to come to that temperature. Prior to use, the test cultures were passed through a series of three daily transfers in plain broth with incubation at 37.5°C. Transfers to the test solutions were made with a standard loop needle. Using the same standard loop, the tubes containing the protein hydrochloride and test organisms were sampled at intervals of 2, 5, 10, 20, and 30 minutes. These loop subsamples were transferred to tubes containing 15 ml of sterilized nutrient agar maintained at 45°C. Immediately thereafter the contents of these tubes were poured into sterile petri dishes and the agar was allowed to solidify. As soon as this occurred the plates were placed in an incubator at 37.5°C for 48 hours. Counts were made using a Quebec colony counting chamber. The counts obtained are listed in Table III.

TABLE III

BACTERICIDAL ACTION OF CRYSTALLINE PROTEIN IN WATER SOLUTION AS SHOWN BY METHOD OF CADE AND HALVORSON

Concentration of protein hydrochloride	Bacterial count per standard loop subsample with *Staphylococcus aureus*						Bacterial count per standard loop subsample with *Eberthella typhi*					
	Exposure period in minutes						Exposure period in minutes					
	0	2	5	10	20	30	0	2	5	10	20	30
mg per ml												
1.0	4,160[1]	58	12	3	3	2	4,820	354	232	107	80	38
0.5	4,160	55	19	7	6	5	4,820	352	182	98	82	49
0.25	4,160	67	23	11	9	4	4,820	224	162	88	34	22
0.10	4,160	72	44	27	19	14	4,820	576	148	87	27	20
0.05	4,160	54	33	24	5	5	4,820	402	308	22	19	19
0.01	4,160	87	18	6	6	1	4,820	4,600	3,600	1,900	1,200	320
0.005	4,160	218	42	24	8	6	4,820	—	—	—	—	—
0.001	4,160	3,420	2,580	2,240	1,870	670	4,820	—	—	—	—	—
None	4,160	3,840	3,710	3,710	3,580	3,560	4,820	4,640	4,610	4,490	4,460	4,460

[1] Each figure is the average of two or more determinations. Zero counts were recorded occasionally but could not be duplicated.

A greater activity is shown against the Gram-positive organism *Staphylococcus aureus* than the Gram-negative organism *Eberthella typhi*. With the former, killing of cells occurred in dilutions as low as 0.005 mg per ml, whereas the lowest concentration which showed appreciable killing with *Eberthella typhi* was 0.05 mg per ml. Also, at equivalent concentrations showing death of cells with both organisms a greater percentage of the cells of *Staphylococcus aureus* was killed than with *Eberthella typhi*.

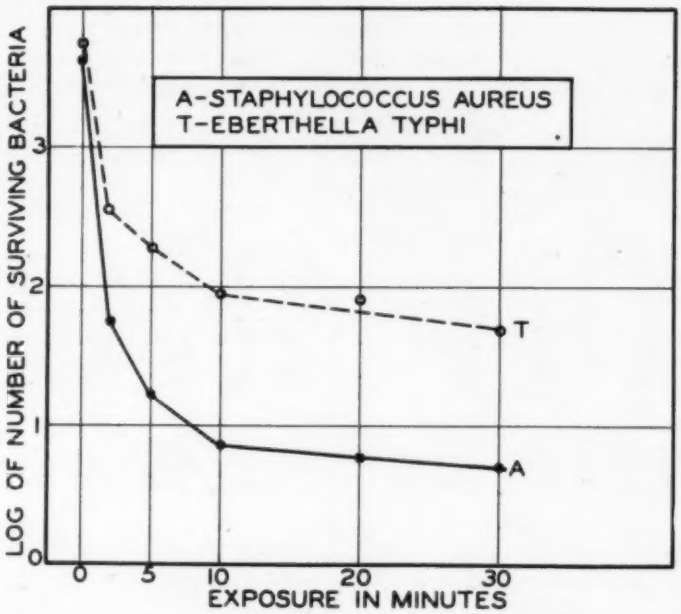

Fig. 2. Logarithmic survival curves of *Staphylococcus aureus* and *Eberthella typhi* in solutions of wheat flour protein at 0.5 mg per ml.

In Figure 2 the logs of the numbers of surviving bacteria after the various exposure intervals to solutions containing 0.5 mg per ml of the crystalline protein hydrochloride are plotted. These curves give a clear picture of the comparative activity of the protein solutions against the two test organisms. It would appear from the data in Table III that the logarithmic survival curves remain the same for each organism over the range of protein concentrations that give any appreciable killing effects. This characteristic is rather unusual.

"In Vivo" Bactericidal Tests

With bactericidal polypeptides Dubos and Cattaneo (1939) were unable to find any positive correlation between "in vitro" and "in vivo" activity. It seems desirable, therefore, to conduct "in vivo"

studies. In these tests *Pneumococci Types I and III, Streptococcus viridans*, and *Streptococcus epidemicus*[4] (Beta type of hemolysis) were used as test organisms. White Swiss mice between eight and ten weeks old were employed. They weighed 20 ± 0.2 g each. All inoculations and injections were made into the peritoneal cavity. With the strains of pneumococci, virulence was first built up by serial passages through mice. Saline suspensions of organisms in peritoneal washings from mice inoculated 18 hours previously were used for

TABLE IV

RESULTS OF "IN VIVO" BACTERIDICAL TESTS

Test organism and minimal lethal dose per mouse	Number of mice inoculated	Size of inoculation per mouse	Amount of protein hydrochloride given	Results[1]					
		ml	*mg*						
Pneumococcus Type I, 0.0001 ml	6	0.2	0	D44	D48	D96	D96	D96	D108
	6	0.2	0.01	D48	D72	D96	D96	D132	D132
	6	0.2	0.02	D36	D36	D44	D72	D72	D96
Pneumococcus Type III, 0.00001 ml	6	0.2	0	D44	D68	D72	D48	D48	D72
	6	0.2	0.01	D44	D44	D84	D44	D44	D68
	6	0.2	0.02	D44	D44	D44	D44	D44	D44
Streptococcus viridans, not virulent even in 0.5 ml quantity	3	0.5	0	All mice survived.					
	3	0.5	0.01	All mice survived.					
	3	0.5	0.02	D72	D84	D96			
Streptococcus epidemicus, 0.001 ml	3	0.3	0	D72	D72	D112			
	3	0.3	0.01	D72	D112	D184			
	3	0.3	0.02	D44	D72	D72			

[1] The letter "D" indicates death, and the number following the hour at which death occurred or as soon thereafter as the animal was found dead.

inoculation of test animals. With the streptococci, inoculations were made directly from 24-hour cultures washed from horse blood agar slants. The toxicity of the protein and the virulence of the four organisms were first determined.

It was found that 0.3 mg per mouse was the minimal lethal dose of the protein. Although those receiving 0.2, 0.1, and 0.05 mg suffered severe shock, they survived. Those receiving 0.02, 0.01, and 0.005 mg showed only very mild reactions and quickly recovered. On the basis of these studies 0.02 and 0.01 mg were the maximum amounts of the protein that could be designated as safe for use in the "in vivo" tests.

[4] The identity of this organism is not certain. It resembled closely a culture of *S. epidemicus* but was a freshly isolated strain from a case of tonsilitis.

Mice were inoculated with the test organisms and immediately thereafter, within two minutes, the protein in physiological saline solution was injected. These tests, therefore, should measure any protective influence of one dose of the protein against an infective dose of the test organisms. The results of these studies are summarized in Table IV.

From the data in Table IV it is apparent that the protein has no "in vivo" bactericidal activity comparable to that found by Dubos (1940) and Dubos and Cattaneo (1939) for the peptide "gramicidin." It would even appear that in the amounts given the test animals were rendered more susceptible to infection. The average death time for mice receiving the protein was shorter with both *Pneumococcus Type I* and *Pneumococcus Type III* and *Streptococcus epidemicus* than with control mice. With *Streptococcus viridans* the only animals in the tests that died were those that received 0.02 mg of the protein.

Studies with True Yeasts

Inasmuch as the protein substance under investigation was found in wheat flour it seemed desirable to determine its effect on baker's or bread yeast. Fulmer, Nelson, and Sherwood's (1921) basal salt-sugar medium (F) [5] was distributed in 100-ml quantities in Erlenmeyer

TABLE V

EFFECT OF PROTEIN ON STRAINS OF *Saccaromyces cerevisiae* IN MEDIUM (F)

Origin of culture	Test No.	Number of cells in inoculum[1]	Dilution plate count per ml—mg of protein hydrochloride added per ml								
			0.1	0.05	0.025	0.01	0.005	0.0025	0.001	0.0005	0.000
		per ml									
Bakery	1	1,780	0	0	0	0	0	3,400	14,800	97,000	203,000
	2	1,780	0	0	0	0	100	2,200	21,700	109,000	219,000
	Average	1,780	0	0	0	0	50	2,800	18,250	103,000	211,000
Brewery	1	5,670	0	0	0	0	0	10	520	31,000	370,000
	2	5,670	0	0	0	0	0	20	730	44,000	660,000
	Average	5,670	0	0	0	0	0	15	625	37,500	490,000
Winery	1	7,830	0	0	0	0	0	0	800	180,000	290,000
	2	7,830	0	0	0	0	0	0	210	210,000	340,000
	Average	7,830	0	0	0	0	0	0	510	195,000	310,000

[1] Determined by dilution plate method using wort agar.

flasks of 200 ml capacity and sterilized in 15 pounds of steam for 20 minutes. Amounts of the protein hydrochloride were added to give a series of concentrations equivalent to 0.1, 0.05, 0.025, 0.01, 0.005, 0.0025, 0.001, and 0.0005 mg per ml. Each of these flasks and of the control flasks of medium without added protein was then inoculated

[5] Water 1,000 ml; NH_4Cl 1.88 g; K_2HPO_4 1.0 g; $CaCl_2$ 1.0 g; sucrose 100.0 g; pH 5.5.

with 1 ml of a physiological saline suspension of cells of *Saccaromyces cerevisiae* (bakers' strain). All flasks were incubated for 24 hours at 30°C. Dilution plate counts were then made using wort agar. These plates were incubated for 72 hours at 30°C prior to counting.

Similar studies were made with two other strains of *Saccaromyces cerevisiae* used industrially—one by a brewery and the other by a winery. The results of these studies are given in Table V.

The counts in Table V show that the protein is extremely toxic to the strains of *Saccaromyces cerevisiae* used. It apparently killed the inoculum in concentrations of 0.005 mg per ml and higher. It prevented any appreciable multiplication of the inoculum in concentrations as low as 0.001 mg per ml. It would appear, therefore, that this material is considerably more active in killing and inhibiting yeast growth than bacterial growth. For, although 0.005 mg per ml killed a high percentage of the cells of *Staphylococcus aureus*, it did not kill them all and it demonstrated little if any killing action against *Eberthella typhi*. In low concentration, then, this material might be described as a specific yeast poison.

Studies with Pathogenic Fungi of a Yeast-like Nature

In view of the activity of this protein in killing and inhibiting yeast growth, tests were made to determine whether it would demonstrate a like activity against the so-called pathogenic yeasts such as *Debaryomyces nadiformis* (torula histolytica) and *Endomycopsis albicans* (monilia albicans).

Exactly the same technique was employed in these studies as with *Saccaromyces cerevisiae* except that medium (F) was modified to contain 10 g of malt extract and 2 g of bacto-peptone per liter. Each

TABLE VI

EFFECT OF PROTEIN ON GROWTH OF *Debaryomyces nadiformis* AND *Endomycopsis albicans*

Test organism	Test no.	Number of cells in inoculum	Dilution plate count per ml—mg of protein hydrochloride added per ml								
			0.1	0.05	0.025	0.01	0.005	0.0025	0.001	0.0005	0.000
		per ml									
Debaryomyces nadiformis	1	88,000	0	0	0	0	0	0	0	4,400,000	18,400,000
	2	88,000	0	0	0	0	0	0	0	1,080,000	11,100,000
	Average	88,000	0	0	0	0	0	0	0	2,740,000	14,750,000
Endomycopsis albicans	1	99,000	0	0	19	82	120	700,000	16,000,000	35,200,000	41,400,000
	2	99,000	2	3	11	60	260	500,000	12,000,000	19,100,000	37,600,000
	Average	99,000	1	1.5	15	71	190	600,000	14,000,000	27,150,000	39,500,000

flask was inoculated with 1 ml of a saline suspension of cells washed from seven-day wort slant cultures. The results of these studies are given in Table VI. The data given in Table VI show that there is a marked toxicity for these two pathogenic yeasts. *Debaryomyces nadiformis* is completely killed at concentrations of 0.001 mg per ml and above. *Endomycopsis albicans*, although not completely killed, showed very low counts at concentrations of 0.005 mg per ml and higher, indicating that the protein had a definite killing activity for most of its cells.

Studies with Mycelial Fungi

To determine if the toxicity for true yeasts and pathogenic fungi of a yeast-like nature could be interpreted as a general fungicidal activity, tests were made using strains of the common mycelial fungi *Aspergillus niger* and *Rhizopus nigricans*. For these tests potato infusion-dextrose agar at pH 5.6 was employed. This agar was bottled in 100-ml quantities and sterilized. It was then cooled in a constant-temperature water bath to 45°C. As soon as the agar had come to this temperature, the protein hydrochloride was added to bring its concentration in the various bottles to 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, and 0.005 mg per ml. Plates made from the agars containing varying amounts of the protein hydrochloride and control plates made with agar containing no added protein were then inoculated in the center with a few condiospores of the test organisms. All plates were incubated at 30°C.

The results of these studies showed no fungicidal action as far as the mycelial fungi used are concerned. Thus fungicidal activity with this protein would appear to be confined to yeasts and yeast-like organisms.

Lytic Activity

In the nutrient-broth bactericidal tests with this protein the only organism completely killed was *Sarcina lutea*, a bacteria notorious for its susceptibility to certain naturally occurring lytic agents. Thus it seemed essential to determine if the ability of this protein to kill bacteria and yeast cells could be directly correlated with an ability to dissolve the cell walls. Consequently, "in vitro" studies were made with red blood cells (equine); with bacterical cells, *Sarcina lutea;* and with yeast cells, *Saccaromyces cerevisiae*. The red blood cells were separated from defibrinated blood by centrifugation and washed three times in physiological saline prior to use. *Sarcina lutea* was grown on plain agar slants for 48 hours. The cells were then washed with saline and separated by centrifugation. They were also washed three times before being used. The cells of *Saccaromyces cerevisiae* were taken

from 72-hour wort-agar slants, separated and washed for use in the same manner as employed with *Sarcina lutea*.

All three types of cells were suspended in 1.0-ml quantities (of wet mass) in tubes containing 9.0 ml of physiological saline with 0.1, 0.05, 0.025, 0.01, and 0.005 mg per ml of added protein hydrochloride. All tubes containing red blood cells, bacterial cells, and yeast cells suspended in these solutions were incubated at 37.5°C for six hours. They were then examined macroscopically and microscopically for signs of cell lysis. In no case was there any evidence of lytic activity. The results make it apparent that although this protein may have surface activity which will aid a lytic agent when present, it is not a lytic agent in itself and brings about the death of cells by some other means.

Discussion

It has been known for a long time that in cereals some substances are present which prevent fermentation by yeasts. It has been stated that as early as 1895 Jago reported that added wheat flour would inhibit beer-yeast fermentations. Hoffman (1907) attributed this inhibition of yeast activity to substances having the characteristics of albuminous breakdown products. Lindet (1910), Lange (1907), and Henneberg (1908) found that both wheat and rye contained substances that actually killed beer yeast. Baker and Hulton (1910), Lecourt (1928), Hayduck (1909), and Mohs and Kühl (1929) all attempted to separate or to define indirectly the substance in wheat flour possessing this activity. Their results showed in general a material having activity in very high dilution with toxic amine characteristics.

From the results of the studies reported in this present paper it would appear that the protein separated and crystallized as the hydrochloride by Balls, Hale, and Harris (1942) is in all probability the substance that gives wheat flour its yeast-killing activity. It is active in very high dilutions. It is strongly basic in nature, containing 20% of the basic amino acid arginine. Thus, its activity might easily be interpreted as that of a toxic amine when studied in unpurified preparations.

This protein in flour and meals has at times caused serious trouble to millers and bakers since, if present in high concentration, it acts to prevent or interfere with the rising of bread dough. The separation and purification of this material appear to constitute a major step toward the control of this problem. If, as seems likely, rye and barley contain the same or similar proteins the development of laboratory and industrial methods for removal or inactivation of such proteins should interest both distillers and brewers.

It is of special interest to note that Lecourt (1928), working with samples of wheat flour, found that the yeast-poisonous principle present had no influence on yeast zymase although it killed the cells. Should this prove to be the case with the purified protein used in these studies, this protein should serve as a valuable tool for the enzyme chemist in his studies on the enzyme complexes of yeast cells.

The activity in killing the pathogenic yeast *Endomycopsis albicans* suggests a possible therapeutic use for this protein in the treatment of tropical sprue. It might also have value in the treatment of blastomycosis where yeast-like organisms are involved. Further, its potential value as a fermentation control or aid where yeast growth is undesirable should not be overlooked.

It should be emphasized that the experiments reported herein are exploratory in nature and that results obtained under conditions differing from those specified may not be similar. A great deal remains to be done relative to the effects of pH, electrolytes, and organic materials on the activities reported herein.

Summary

Studies of the bactericidal and fungicidal activity of a protein separated from unbleached wheat flour have been made, using the protein in the form of the purified, crystallized hydrochloride. This protein has both bactericidal and bacteriostatic activity "in vitro." No "in vivo" activity could be demonstrated when tested in mice against pneumococci and streptococci. "In vitro" activity was greatest against Gram-positive organisms but there was also some activity against Gram-negative organisms.

When tested against *Saccaromyces cerevisiae* this protein was also found to possess a marked fungicidal activity, bringing about the death of the yeast cells in concentrations of 0.005 mg per ml and higher. Similar results were obtained with the pathogenic fungi *Debaryomyces nadiformis* and *Endomycopsis albicans*. However, tests using mycelial fungi failed to show any fungicidal activity. Thus, the fungicidal activity of this protein seems to be restricted to yeasts and yeast-like types of fungi.

"In vitro" tests failed to show any hemolytic, bacteriolytic, or yeast-cell lytic action. Thus, this protein cannot be classified as a lytic agent.

The industrial significance of this protein to millers, bakers, brewers, and operators of industrial fermentations has been discussed briefly.

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EFFECT ON SMALL LABORATORY ANIMALS OF THE INJECTION OF THE CRYSTALLINE HYDROCHLORIDE OF A SULFUR PROTEIN FROM WHEAT FLOUR *

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In previous papers from the Enzyme Research Laboratory Balls and Hale (1940) reported the occurrence of a sulphydryl compound of protein-like nature in the petroleum-ether extract of wheat flour and Balls, Hale, Harris (1942) reported the chemical examination of a crystalline protein obtained from this petroleum-ether extract. More recently Stuart and Harris (1942) showed that the crystals (as well as the partly purified material from which they were obtained) had a powerful killing action against some, but not all, species of bacteria.

In view of the toxicity toward bacteria, it is of interest to learn if animals could be protected by this substance against inoculation with disease-producing microörganisms, as Dubos and Cattaneo (1939) succeeded in protecting mice against pneumococcus by injections of gramicidin. It was found that the material derived from wheat is itself toxic to animals when injected either intraperitoneally or intravenously. When given by mouth, however, relatively large doses were found to be harmless. The behavior of the substance is thus similar to what has been frequently observed in other bodies of protein-like structure and relatively low molecular weight, such as protamines and peptones.

The symptoms observed after injection were usually labored respiration, followed (especially in mice) by loss of equilibrium. This was succeeded by an apparent coma, which may have been paralysis with the muscles relaxed. Thereafter death or recovery occurred. In rabbits and guinea pigs, however, a large but sublethal dose sometimes produced no symptoms at all, whereas mice usually showed distress with such doses although they eventually recovered. Because most gross symptoms of toxicity other than death cannot be tabulated satisfactorily, the reaction following injection has been described in the tables merely as none, slight, moderate, or severe.

The activity of the crystalline hydrochloride was further studied by the Schultz-Dale technique on the isolated uteri of virgin guinea pigs. It was found that the substance contracted the normal uterus, tracing

* Joint contribution from the [1] Allergen Investigations Division and the [2] Enzyme Research Laboratory of the Bureau of Agricultural Chemistry and Engineering. Work done under Special Research Funds provided by Bankhead-Jones Act.

a curve similar to that obtained by Rocha e Silva (1940) with trypsin. Unlike trypsin, however, doses of the protein did not desensitize the muscle to subsequent doses thereof. The sensitivity of the uterus to the protein was greatest in Tyrode solutions containing 0.025 to 0.05 g calcium chloride per liter. Contraction was usually slight or entirely inhibited in calcium-free Tyrode or in solutions containing 0.075 g or more of calcium chloride per liter. The smallest dose of the protein to contract the uterus maximally was found to be of the order of 1 part in 1,250,000. Onset of contraction was slower than that produced by small doses of histamine but contraction was sustained much longer with the protein. The present paper reports the experiments on which the foregoing statements are based.

Experiments on mice: Swiss white mice[1] between eight and ten weeks old and weighing 20 g each were injected intraperitoneally with

TABLE I

INTRAPERITONEAL INJECTION OF MICE WITH SOLUTION OF THE CRYSTALLINE HYDROCHLORIDE

Amount injected	Reaction following injection
mg	
0.50	Died in 40 minutes.
0.30	Died in 60 minutes.
0.20	Severe; recovered in 2 hours.
0.10	Severe; recovered in 18 hours.
0.050	Severe; recovered in 18 hours.
0.010	Slight; recovered in 18 hours.
0.005	Slight; recovered in 18 hours.

a solution of the crystalline hydrochloride in physiological salt solution. The volume injected varied between 0.05 ml and 0.5 ml. The results given in Table I refer individually to each animal used and show the weight of crystals received by that animal.

A similar experiment was then made using material that had been hydrolyzed by acid as is commonly done with proteins. 10.6 mg of protein hydrochloride in 0.3 ml of 20% HCl was boiled under a reflux condenser for 5 hours. The liquid was then neutralized with sodium bicarbonate and diluted to 10 ml with water. Appropriate amounts were injected with the results shown in Table II.

From these experiments, it appears that the minimum lethal dose of the original hydrochloride for mice is about 15 mg per kilo of body weight when injected intraperitoneally. Acid hydrolysis for five hours greatly reduced the toxicity, but did not altogether destroy it.

Experiments on guinea pigs and rabbits: The results of intraperitoneal injection in guinea pigs and rabbits are given in Table III for

[1] We are indebted to Mr. L. S. Stuart of this Bureau for supervising the experiments with mice.

TABLE II

INJECTION OF MICE WITH THE SUBSTANCE AFTER ACID HYDROLYSIS

Amount injected	Reaction following injection
mg	
1.00	Severe and immediate; complete recovery in 18 hours.
0.60	" " " " " " 18 "
0.60	" " " " " " 18 "
0.50	" " " " " " 18 "
0.50	" " " " " " 18 "
0.30	Severe in 20 minutes; " " " 18 "
0.30	" " 20 " " " " 18 "
0.20	" " 20 " " " " 18 "
0.20	" " 20 " " " " 18 "

each animal used. The preparation injected was an aqueous solution neutralized to litmus with sodium hydroxide. The volume injected varied between 0.25 and 0.50 ml. The experiments are not sufficient in number to permit an estimate of the fatal dose, but they do indicate that for guinea pigs this dose is of the same order of magnitude as for mice. Rabbits appear to be somewhat more resistant.

TABLE III

INTRAPERITONEAL INJECTION OF GUINEA PIGS AND RABBITS

Weight of animal	Dose	Reaction following injection
g	*mg per kilo of body weight*	
	GUINEA PIGS	
304	6.6	None observed.
346	5.8	None observed.
365	5.5 + 5.5 in 17′	Moderate distress; died 2 days later.
318	6.3 + 6.3 in 4′	None observed; died following day.
320	12.8	None in 1 hour; died following day.
354	11.4	None observed.
	RABBITS	
2136	0.7	None observed.
1528	5.2	None observed.
1749	8.9	None observed.
1595	13.8	Slight, normal in 9′.
1410	12.0	None observed.

Table IV shows the results of intravenous injection in rabbits and guinea pigs. The material was dissolved in water and the solution neutralized to litmus with sodium hydroxide. The concentrations were so chosen that the volume of liquid injected into the animal was 0.2–0.4 ml. It is evident that an intravenous dose of 1.6 mg per kilo of body weight would be fatal to guinea pigs in about half the cases. Rabbits are probably more resistant than guinea pigs but the number

TABLE IV

INTRAVENOUS INJECTION OF GUINEA PIGS AND RABBITS

Weight of animal	Dose	Reaction following injection
g	*mg per kilo of body weight*	
	GUINEA PIGS	
330	4.7	Dead in 1 minute.
304	4.0	Dead in 1 minute.
335	3.7	Dead in 10 minutes
316	2.2	Dead in 10 minutes.
415	2.2	Dead in 10 minutes.
367	2.2	Dead in 5 minutes.
314	1.7	Severe; recovery complete.
402	1.6	Dead in 17 minutes.
336	1.6	Severe; recovery complete.
330	1.6	Dead in 14 minutes.
412	1.1	Moderate; recovery complete.
363	1.1	Slight; recovery complete.
324	1.1	Slight; recovery complete.
350	1.1	Moderate; recovery complete.
370	1.1	Moderate; recovery complete.
	RABBITS	
1562	12.5	Dead in 10 minutes.
1810	4.6	Dead in 9 minutes.
1064	4.5	None observed.
1750	2.9	None observed.
1498	2.5	Severe; died overnight.
2000	3.0	None observed.

TABLE V

ORAL ADMINISTRATION AND SUBSEQUENT INTRAVENOUS INJECTION

Initial weight of animal (*g*)	349	309	387
Amount given:			
(*mg*)	80	32	40
(*mg/kilo*)	229	104	103
Symptoms within 7 days of ingestion	none	none	none
Dose injected 7 days after oral administration (*mg/kilo*)	2.6 (intraven.)	1.0 (intraven.)	19.5 (intraperit.)
Reaction following injection	Dead in 5 minutes	Slight	None observed
Final weight (*g*)	361	327	390

of animals used was too small to permit an approximation of the fatal dose.

Oral administration: Relatively large doses of the crystalline substance (dissolved in water and neutralized with NaOH to litmus) were given to guinea pigs through a stomach tube without any apparent effect within a week. The doses were somewhat greater than 50 to 100 times the fatal intravenous dose, as shown by the preceding experiments. In each case the animals gained weight and appeared

to be quite normal. They were, however, still sensitive to intravenous injection of the same material.

Experiments with excised guinea pig uterus: The Schultz-Dale apparatus used was a modification of the apparatus employed for the assay of posterior-pituitary solutions by the U. S. Pharmacopoeia method. The volume of each bath was 50 ml. Tyrode solution with reduced calcium content, as frequently employed in anaphylactic studies, made possible the use of uteri from mature animals. On the

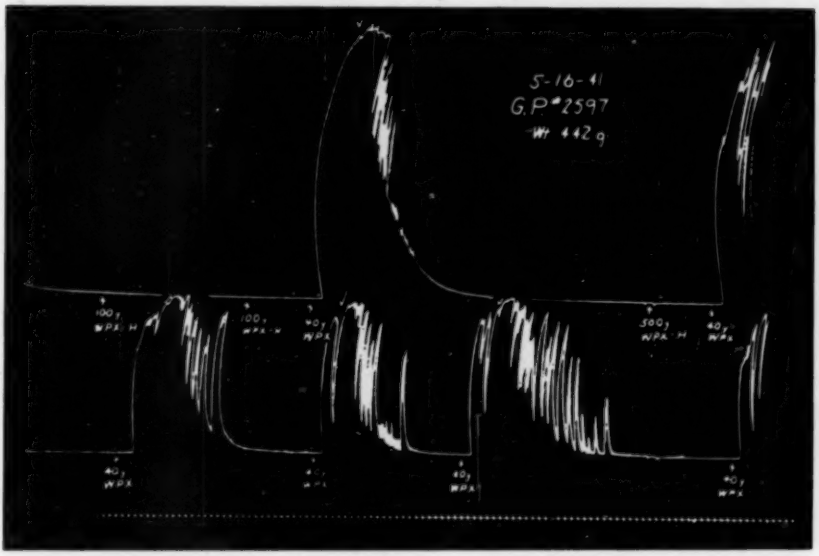

Fig. 1. Showing the effect of acid hydrolysis on the uterus-contracting property of WPX; also repeated contractions with equal quantities of WPX. The check marks (✓) above the curves indicate the point at which the bath was flushed and fresh Tyrode solution added. Time interval is in minutes.

records, reproduced as Figures 1 to 4, the symbol WPX has been used to designate the protein-like substance from wheat; the other abbreviations are self-explanatory. The amount of substances noted on the records represents the total added to the 50-ml baths.

In Figure 1 the lower curve represents the contraction of one horn of the uterus with 40 micrograms (1 part in 1,250,000) of WPX repeated three times with the same quantity of material. It is evident that the muscle does not become desensitized to WPX. The upper curve of Figure 1 shows the effect on the second horn of the uterus of WPX that has been hydrolyzed 5 hours with 20% HCl (WPX-H). Thus acid hydrolysis completely destroys the muscle-contracting property of the substance.

Large doses of the substance produce an indefinitely sustained contraction of the uterus if the bath is not flushed with fresh Tyrode

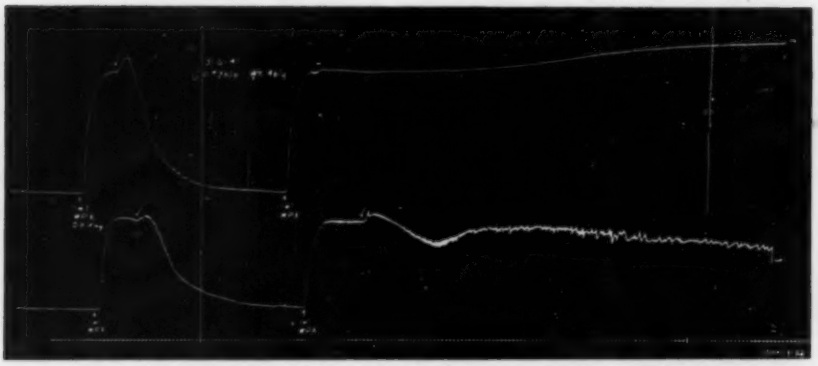

Fig. 2. Showing sustained contraction of the uterus with large doses of WPX. 1 ml solution contained 0.16 mg WPX.

solution soon after contraction has reached a maximum. This effect is shown in Figure 2.

Figure 3 shows the effect of the calcium ion concentration on the contractility of the uterus with the peptide. Concentration of calcium chloride in the Tyrode solution above 0.075 g per liter apparently inhibits contraction. Likewise contraction is inhibited in calcium-free Tyrode solution. In other experiments contraction of the uterus was

Fig. 3. Influence of calcium content of Tyrode solution on contractility of uterus by WPX.

frequently inhibited in Tyrode solutions containing 0.075 g calcium chloride per liter (Fig. 4). It has also been noted that uteri failing to contract with WPX when suspended in solutions of high calcium content will go into a sustained contraction as soon as the bath is flushed with Tyrode solution containing 0.025 g of $CaCl_2$ per liter.

It is well known that anaphylactic and histamine contraction will occur in Tyrode solutions over the whole range of calcium concentration from calcium-free solutions to those containing the usual quantities (0.2 g of $CaCl_2$ per liter). Since contraction of the uterus with the protein-like substance from wheat seems to be blocked in the

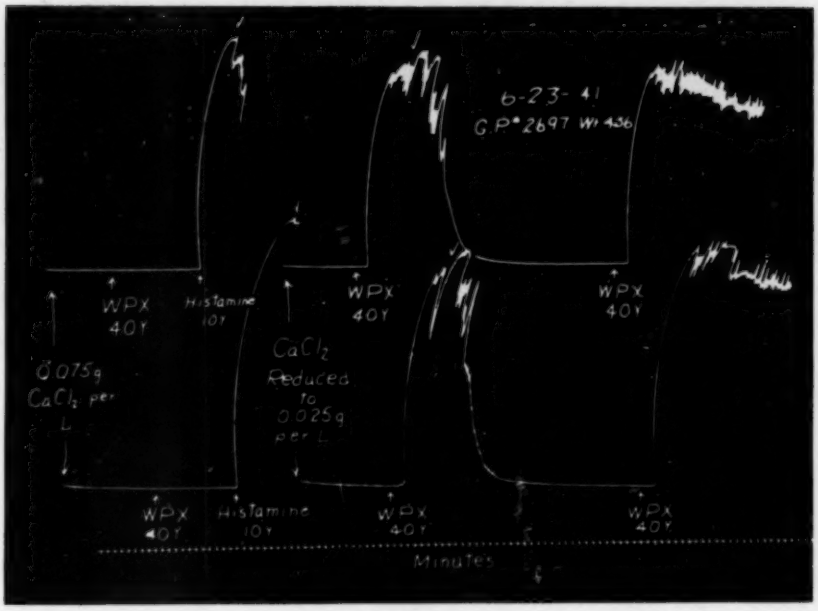

Fig. 4. Influence of calcium content of Tyrode solution on contractility of uterus by WPX.

higher ranges of calcium concentrations (and in calcium-free solutions) it would appear that either the reaction is not due to the liberation of histamine from the tissue or the reaction leading to the liberation of histamine is inhibited by the higher calcium ion concentration.

The failure of desensitization noted in the present experiments suggests the possibility that the site of action of the protein is not identical with that of trypsin, which is said by Rocha e Silva (1941) to desensitize the muscle.

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THE VARIABILITY IN THE THIAMIN CONTENT OF WESTERN CANADIAN HARD RED SPRING WHEAT OF THE 1940 CROP

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The Ogilvie Flour Mills Co., Ltd., Montreal, Canada

(Read at the Annual Meeting, May 1941)

In literature on the subject there are many references to the " whole-wheat level " of thiamin in flour or other wheat products. This " level " is generally considered to be 1.65 International Units of thiamin per gram or 4.95 μg per gram. The word " level " implies that this figure is the average and that individual values vary only slightly from the mean.

In recent years several investigators have presented data which indicate that the thiamin content of wheats may vary within a wide range. Baker and Wright (1935) obtained values of 6.9 to 10.2 μg per g; Morgan and Hunt (1935), 3.75 and 5.67; Baker, Wright, and Drummond (1937), 3.6 to 7.8; Leong and Harris (1937), 4.5; Copping and Roscoe (1937), 3.54. Booth (1940) found that commercial samples of wheat from various parts of the world ranged from 1.62 to 7.80 μg of thiamin per gram with a mean value of 3.75. Wheats grown in England were slightly higher than this world average, varying from 2.34 to 5.94 with a mean value of 4.17. A series of 31 American wheats of various types reported by Schultz, Atkin, and Frey (1941) ranged from 4.2 to 7.3 μg, with a mean value of 5.6.

The present study was planned to determine the average thiamin content of Canadian hard red spring wheat and the variability that might be expected.

Experimental

Two hundred and sixty-five samples of hard red spring wheat of the 1940 crop were used for this study. They were samples of car lots from shipping points in Manitoba, Saskatchewan, and Alberta and were selected so as to cover this area as well as possible with a limited number of samples. The wheats were commercially graded from No. 1 Northern to No. 6 Northern, but only 14% of the samples graded lower than No. 2 Northern. The principal degrading factors in the case of the 14% were the presence of rain-bleached, starchy, frosted, or immature kernels. The weights per bushel ranged from 56 to 65 pounds, but 93% of the samples tested better than 60 pounds. Most of the samples were, therefore, sound heavy wheats.

Because the samples were taken from regular commercial car lots it was impossible or impractical to segregate them by variety. However, they were chiefly Thatcher with some Marquis, Renown, and Red Bobs. The latter variety was grown chiefly in Alberta.

Their thiamin content was determined by a thiochrome method described by Johannson and Rich (1941). Moisture, ash, and protein determinations were made according to the official A.A.C.C. methods. All the data were calculated to a 13.5% moisture basis.

The thiamin content of the samples is presented in the form of a map in Figure 1. The origin of each sample is shown as a dot and samples of similar thiamin content are designated by distinctive markings. Four groupings were chosen as indicated in the legend.

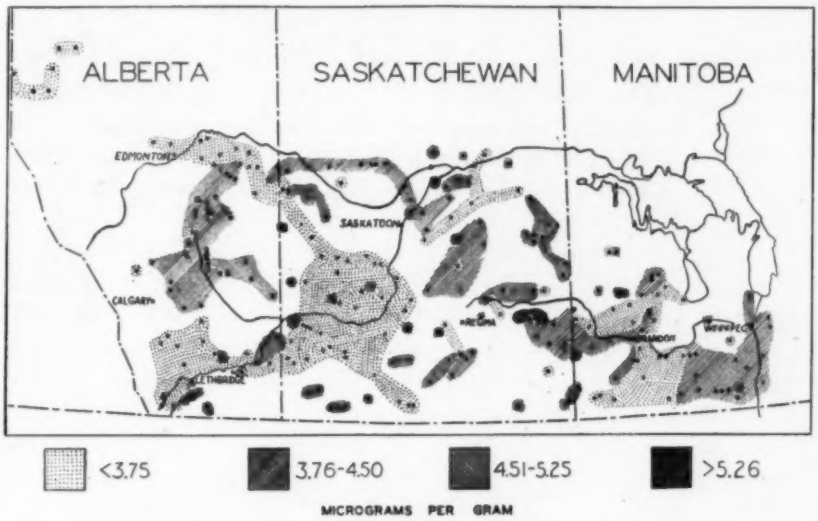

Fig. 1. Areas with wheats of similar thiamin content.

It is realized that to make a complete survey of an area as large as western Canada would require several thousand samples, but the volume of data is sufficient to indicate probable trends. From an inspection of the map it would appear that certain areas produce wheat of high thiamin content and that other sections produce low-thiamin wheat, but the areas are too scattered for definite conclusions. In many cases, samples of very high and very low thiamin content were found in adjacent areas.

Maps were available which showed western Canada divided into definite areas according to general soil type, and also into areas based on the ash and protein content of the wheat that each produced, but in no case did the thiamin distribution follow the outlines of any of these areas.

The summarized statistics are presented in Table I and graphically in Figures 2 and 3.

The means and range of values are almost identical with the data given by Booth (1940) on world wheats, but they are lower than those given for United States wheats by Schultz, Atkin, and Frey (1941).

TABLE I

STATISTICS ON THE THIAMIN DATA ACCORDING TO PROVINCE

Values expressed as micrograms per gram on a 13.5% moisture basis

Province	No. of samples	Mean value	Standard deviation	Range
		μg/g	μg/g	μg/g
Manitoba	67	3.90	0.606	3.0–5.3
Saskatchewan	120	3.99	0.708	2.5–6.2
Alberta	78	3.90	0.909	2.2–8.0
All provinces	265	3.93	0.750	2.2–8.0

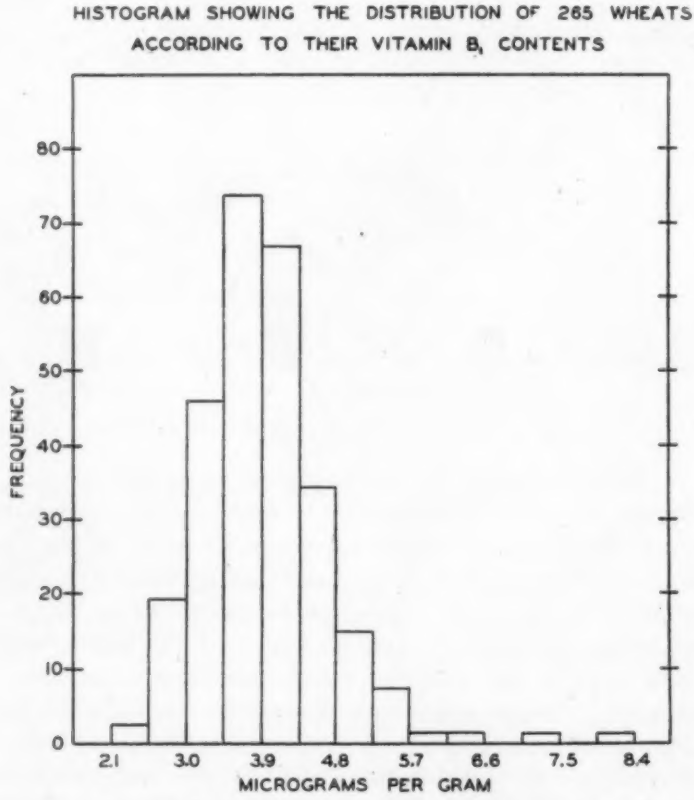

Fig. 2. The distribution of 265 wheats according to their vitamin B_1 contents.

There is no significant difference in the means of the three provinces, but the Alberta samples covered a considerably greater range in thiamin values.

The factors responsible for this large variability in thiamin content are not yet known. Data given by Booth (1940), Nordgren and

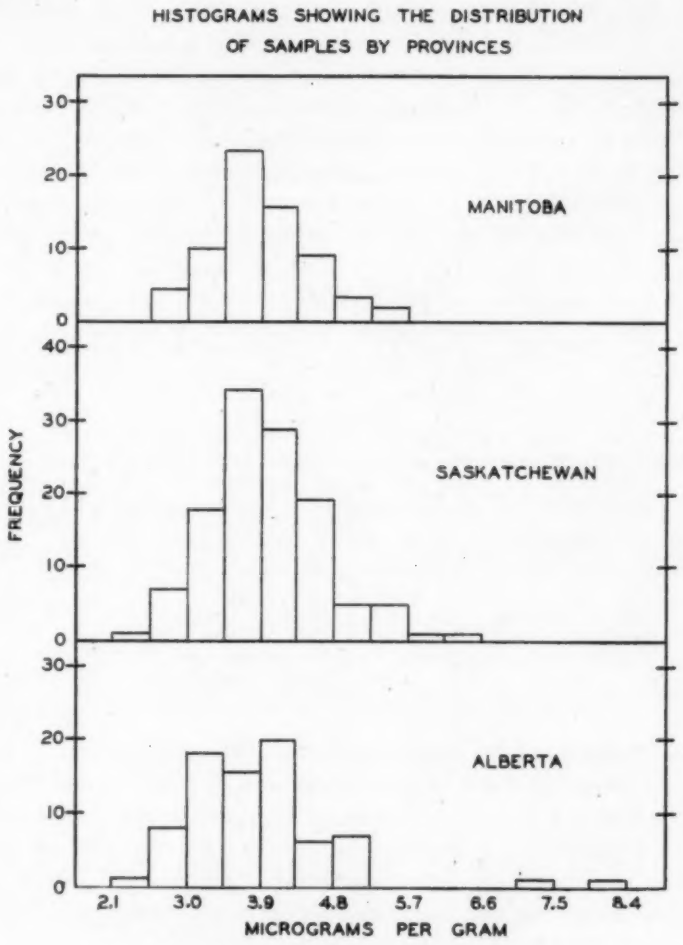

Fig. 3. The distribution of samples by provinces.

Andrews (1941), and Newman (1941) indicate that variety and environment are influencing factors. Burkholder and McVeigh (1940) while studying the growth of two corn hybrids in nutrient media found that the thiamin content of the kernels varied directly with the nitrogen supply and inversely with the phosphorus in the nutrient solution. This

indicates that composition of the soil may play an important part in determining the vitamin B_1 content of the kernels. It is known that western Canadian soils vary greatly in both nitrogen and phosphorus content. This fact may account in part for the present data.

It is generally known that in a series of flours of varying grades, or within a group of mill-stream flours, there is a high correlation between thiamin content and both ash and protein content. The correlation coefficient of thiamin and the ash content in this series was + .012, and between thiamin and protein content + .161. The 5% point in both cases was + .195. Both values indicate no relationship for this series.

No relation could be found between thiamin content and weight per bushel or grade. Commercial wheats are degraded for numerous reasons—for example, because of the presence of immature, frozen, thin and shrunken, starchy, or otherwise damaged kernels. Unfortunately, this series was not suited to a study of these factors because most of the samples were sound and of good weight. Two samples, however, containing different degrading factors were hand picked and the normal and damaged kernels assayed separately.

TABLE II

THE THIAMIN CONTENTS OF THE NORMAL AND DAMAGED FRACTIONS OF TWO WHEAT SAMPLES

Sample No.	Commercial grade	Weight per bushel	Fraction	Thiamin content
		lbs		*μg per g*
86	No. 6 Northern	56	Normal kernels	3.4
			Thin and shrunken kernels	4.2
115	No. 3 Northern	64	Normal kernels	3.5
			Starchy kernels	2.9

The results obtained with sample No. 86 were anticipated because thin and shrunken kernels have a higher ratio of bran to endosperm than do normal plump kernels. The starchy kernels from sample No. 115 were somewhat lower in thiamin content than the normal kernels. Because the two fractions were grown under identical circumstances this may be an indication that conditions which produce high protein content may also produce high thiamin content.

Summary

Two hundred and sixty-five commercial hard red spring wheats from most of the crop districts in western Canada were assayed by the thiochrome method. The values ranged from 2.2 to 8.0 μg of thiamin per gram with an average of 3.93 μg per gram. The means for each prov-

ince were not significantly different, but the western part of the area studied showed a greater range in values.

The areas producing wheats of similar thiamin content appeared to have a random arrangement. No relation to soil type was observed.

The large variability observed is undoubtedly due to the influence of factors such as soil composition, climatic conditions, wheat variety, etc., and since the geographical location of the samples in this series were the only information available, these points could not be investigated. For this purpose several series of controlled experiments would be necessary to evaluate the influence of each factor on the thiamin content of wheat.

Acknowledgment

The authors are indebted to Dr. J. A. Anderson of the Dominion Grain Research Laboratory for his kind assistance in supplying many of the samples used in this study.

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BOOK REVIEW

The Chemical Formulary, Volume V. Edited by H. Bennett. Published by the Chemical Publishing Co., Brooklyn, N. Y. 674 pages. $6.00.

An examination of this book gives no reason to doubt the statement in the preface that "sufficient new formulae have been gathered to compile a 5th volume . . . which will broaden and bring up-to-date the contents of Volumes I, II, III, and IV."

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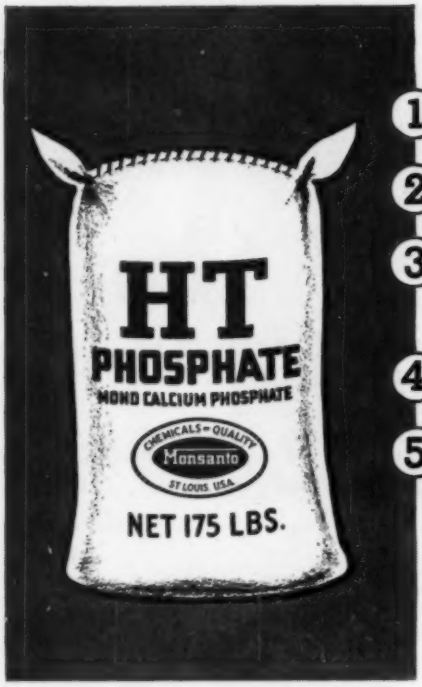

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